VOLUME 69 • MARCH 1960 • NUMBER 3

PATHOLOGY

Official Organ for the AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY

AMERICAN MEDICAL ASSOCIATION PUBLICATION

TABLE OF CONTENTS

VOLUME 69 MARCH 1960 NUMBER 3

ORIGINAL ARTICLES

REGULAR DEPARTMENTS

A. M. A.
ARCHIVES of PATHOLOGY

Official Organ for the AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY

VOLUME 69 MARCH 1960 NUMBER 3

Published under the Auspices of the Board of Trustees

The A. M. A. Archives of Pathology is published monthly by the American Medical Association and is an official publication of the Association.

Executive Vice President: F. J. L. BLASINGAME, M.D.
Business Manager: RUSSELL H. CLARK

ADVERTISING: Advertising Manager, John L. Murphy; Advertising Production Manager, Walter H. Kimotek. Advertising Representatives: Vernon J. Wendt, 535 North Dearborn Street, Chicago 10, Illinois, WHitehall 4-1500; Ronald Davis, 475 Fifth Avenue—Room 1005, New York 17, New York, ORegon 9-9383; Dale B. Clarke, 1919 Wilshire Blvd., Los Angeles 57, California, HUbbard 3-3811.

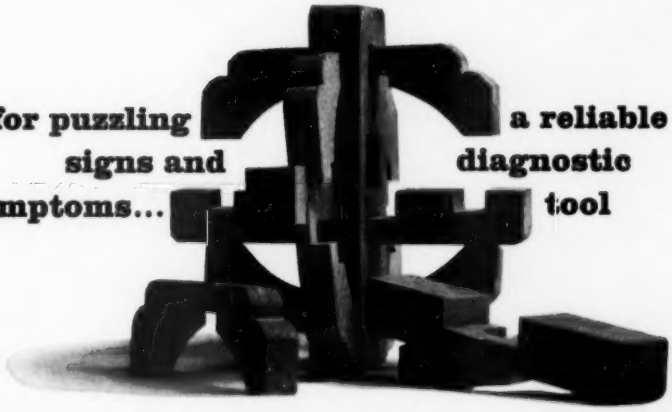
CIRCULATION: Circulation Manager, Robert A. Enlow; Assistant Circulation Manager, Bernard F. Kroeger. The subscription rate of the A. M. A. Archives of Pathology is: United States, U. S. Possessions, and Canada, one year, $8.00; two years, $12.00; all other countries, one year, $10.00; two years, $16.00. Special price to Residents, Interns, and Medical Students in the United States, U. S. Possessions, and Canada, one year, $4.00; two years, $6.00. Single copies of the current year and the immediate past year will be $1.00 each and may be ordered from the Circulation Department, American Medical Association, 535 North Dearborn Street, Chicago 10, Illinois.

Back issues older than two years are available through Walter J. Johnson, Inc., 111 Fifth Avenue, New York 3, New York.

CHANGE OF ADDRESS: Please notify publisher at least six weeks in advance, including both old and new address, and mailing label taken from the most recent copy.

Second-class postage paid in Nashville, Tennessee.

Please address communications and subscription orders to: American Medical Association, 535 North Dearborn Street, Chicago 10, Illinois.



for puzzling signs and symptoms...

a reliable diagnostic tool

When signs and symptoms refuse to fall into place, the C.R.P.A. test accurately indicates inflammatory and necrotic diseases. A simple office procedure taking less than two minutes to set up and perform, the C.R.P.A. test can be used *prior to other tests* to determine the necessity for an erythrocyte sedimentation rate or SGO-T level.

Unlike the ESR or SGO-T level, a C.R.P.A. test demonstrates no variability in normal values. It is influenced only by the C-reactive protein in the patient's blood. A positive reaction always indicates pathology;[1] no false positives have ever been reported.

The C.R.P.A. test is a consistently constant indicator of acute myocardial infarction,[1-9] acute rheumatic fever,[10-12] active widespread malignant disease,[1,6,7,12,13] and bacterial infections.[1,5,7,14]

C·R·P·A® *test*

for routine blood chemistry

The reliability of the C.R.P.A. test makes it invaluable in differential diagnoses and routine work-ups. It may be used to deny or confirm ESR and SGO-T readings. Because it is so sensitive it can mark the progress of the disease and measure the effectiveness of therapy.[1,15]

References: 1. Editorial: J.A.M.A. 160:672 (Feb. 25) 1956. 2. Levinger, G. L.; Levy, H., and Elster, S. K.: Ann. Int. Med. 46:68, 1957. 3. Kroop, I. G., and Shackman, N. H.: Am. J. Med. 22:90, 1957. 4. Kroop, I. G., and Shackman, N. H.: Proc. Soc. Exper. Biol. & Med. 86:95, 1954. 5. Shubin, H.; Glaskin, A., and Heiken, C. A.: Tuberc. 15:62, 1955. 6. Losner, S., and Volk, B. W.: New York J. Med. 56:2665 (Sept. 1) 1956. 7. Roantree, R. J., and Rantz, L. A.: A.M.A. Arch. Int. Med. 96:674, 1955. 8. Goldner, F., and Meador, C.: South M. J. 48:1339, 1955. 9. Hedlund, P.: Acta med. scandinav. supp. 196:579, 1947. 10. Shackman, N. H.; Heffer, E. T., and Kroop, I. G.: Am. Heart J. 48:599 (Oct. 13) 1954. 11. Boland, E. W.: California Med. 82:65, 1955. 12. Knights, E. M., Jr.; Hutchins, M.; Morgan, E., and Ploompuu, J.: J.A.M.A. 162:9 (Sept. 1) 1956. 13. Shetlar, M. R.; Bullock, J. A.; Shetlar, C. L., and Payne, R. W.: Proc. Soc. Exper. Biol. & Med. 88:107, 1955. 14. Ruggieri, P. A.: J. M. Soc. New Jersey 52:500, 1955. 15. Dodd, K.: Mississippi Doctor 33:59, 1955.

Complete literature and bibliography available on request.

Schieffelin & Co./Since 1794 Pharmaceutical Laboratories Division, New York 3

Instructions to Contributors

Articles, book reviews, and other materials for publication should be addressed to the Chief Editor. Articles are accepted for publication on condition that they are contributed solely to this journal.

An original typescript of an article, with one carbon copy, should be provided; it must be double or triple spaced on one side of a standard size page, with at least a 1-inch margin at each edge. Another carbon copy should be retained by the author.

The main title of an article may not contain more than eighty characters and spaces; a subtitle may be of any length.

The author's name should be accompanied by the highest earned academic or medical degree which he holds. If academic connections are given for one author of an article, such connections must be given for all other authors of the article who have such connections.

If it is necessary to publish a recognizable photograph of a person, the author should notify the publisher that permission to publish has been obtained from the subject himself if an adult, or from the parents or guardian if a child. An illustration that has been published in another publication should be accompanied by a statement that permission for reproduction has been obtained from the author and the original publisher.

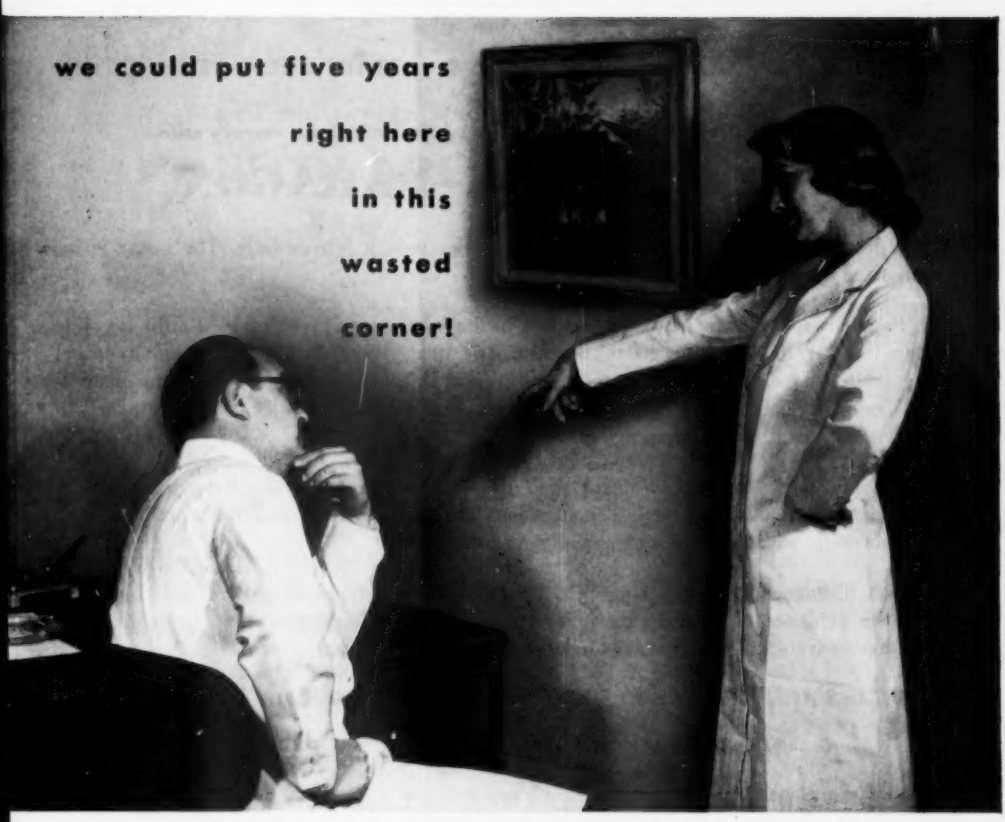
Oversized original illustrations should be photographed and a print on glossy paper submitted. Prints of a bluish tinge should be avoided. Large photomicrograph prints will be reduced in scale unless portions to be cropped are indicated by the author. The author should submit duplicate prints of roentgenograms and photomicrographs with the essential parts that are to be emphasized circled, as a guide to the photoengraver.

Charts and drawings should be in black ink on hard, white paper. Lettering should be large enough, uniform, and sharp enough to permit necessary reduction. Glossy prints of x-rays are requested. Paper clips should not be used on prints, since their mark shows in reproduction, as does writing on the back of prints with hard lead pencil or stiff pen. Labels should be prepared and pasted to the back of each illustration showing its number, the author's name, and an abbreviated title of the article, and plainly indicating the top. Charts and illustrations must have descriptive legends, grouped on a separate sheet. Tables must have captions. ILLUSTRATIONS SHOULD BE UNMOUNTED.

References to the literature should be limited to those used by the author in preparation of the article. They should be typed on a special page at the end of the manuscript. The citation should include, in the order given, name of author, title of article (with subtitle), name of periodical, with volume, page, month—day of month if weekly or biweekly—and year. References to books must contain, in the order given, name of author, title of book, city of publication, name of publisher, and year of publication.

AMERICAN MEDICAL ASSOCIATION

535 North Dearborn Street **Chicago 10**



How many microslides do you average a day? Fifty, maybe?

A Lab-aid stack only 19" square and about five feet high would keep five years' output right at your fingertips.

That's because Lad-aid design gives you 45% more capacity, inch for inch, than conventional filing units. Think of it . . . a single unit section only five inches high, compact enough to fit handily on a desk top, will hold 6500 slides!

And that's not all . . . by interchanging different drawer sizes 1", 2", and 4", you can file Kodachromes, lantern slides, and index cards in the same cabinet. Other special-purpose cabinets (same basic dimensions so they all stack together) let you file fresh slides flat in spread-out trays, or file paraffin blocks in shallow drawers.

The beauty of it all is that you get all these advantages at no greater filing cost-per-slide than ordinary cabinets.

TECHNICON Lab-aid®

laboratory filing system

Bulletin No. 14L-56 tells the story. Let us send it to you.

THE TECHNICON COMPANY, CHAUNCEY, N. Y.

Paragon Tray Drawer Cabinet

Compact

Low Cost

FOR FILING
MICROSCOPIC SLIDES 3 x 1"
KODACHROME TRANSPARENCIES 2 x 2" SLIDES
LANTERN SLIDES (up to 3¼ x 4¼)
PETROGRAPHIC SLIDES

When you purchase a
PARAGON TRAY DRAWER CABINET
YOU PURCHASE FILING SPACE ONLY
NO WASTE SPACE-EVERY INCH USED

U. S. Pat. No. 2,202,047
C101—Tray Drawer Cabinet for 3 x 1 Micro Slides
Capacity 4500—18¾ x 15¾ x 4¼

All Paragon Tray Drawer Cabinets are manufactured in standard sizes so that any number of sections may be interlocked to form one cabinet to accommodate any number of varied slides. The dimensions of the different cabinets are the same as to length and width, varying only in height. The cabinet formed by interlocking may be 18¾ x 15¾; 18¾ x 11 or 18¾ x 5 or it may be a pyramid with the sections varying in width.

C221—Capacity 1500 Slides—18¾ x 11 x 3¾
For Filing KODACHROME TRANSPARENCIES and 2 x 2" SLIDES

SPECIFICATIONS: All Paragon Tray Drawer Cabinets are made of reinforced steel construction, olive green finish. Interlocking device enables several units to be joined into one. Each sectional unit contains removable drawers with hand grip in front and rear. Interlocking steel base obtainable whenever required. **Constructed according to rigid specifications—not merely adapted.**

Address your orders and inquiries to Dept. P.
Manufactured Exclusively by

PARAGON C. & C. CO., Inc. • 2540 Belmont Ave., New York 58, N.Y

A.M.A. ARCHIVES OF
PATHOLOGY

Thirty-Five MM. Film as Mounting Base and Plastic Spray as Cover Glass for Histologic Sections

JOHN PHILLIP PICKETT, H. T., and JOACHIM R. SOMMER, M.D., Durham, N.C.

A method of mounting tissue sections on 35 mm. film instead of glass, and covering them with a hardening plastic spray instead of the conventional glass cover slips, has been developed in this laboratory and tried in various applications during the past five years. The striking advantages of this procedure seem worthy of a preliminary report.

Submitted for publication June 11, 1959.

From the Department of Pathology, Duke University Medical Center.

Great appreciation is due Mr. Carl Bishop, who skillfully prepared the photographs, with the assistance of Mr. John Justice.

Dr. W. D. Forbus, Dr. G. Margolis, Dr. B. Fetter, and Mr. Bernard Lloyd have given valuable suggestions, which are gratefully acknowledged.

Technique

Materials.—With the exception of the film (Fig. 3), the film holder, the reel, the

Fig. 1.—Above center: developing reel. Above right: holder, stainless steel. Center right: section in Kodachrome slide frame. Left: Krylon plastic spray. Below center: holder, as in Figure 4. Below: separator.

Fig. 2.—Sections lined up between separator in the process of being picked up.

plastic spray (Fig. 1), and the separator (Figs. 1 and 2), all necessary equipment can be found in any laboratory doing histologic work.

The 35 mm. film is the Cronar, P-40B leader film made by E. I. DuPont de Nemours and Company, Inc., Photo Products Department, Wilmington 98, Del. It is used as a leader film base for 35 mm. motion picture film. Its strength, clarity, chemical inertness, and low cost make it an ideal base for tissue sections. One hundred feet of this film costs $1.60 and provides for approximately 4,000 sections, the cost amounting to roughly 0.04 cent per section.

The plastic spray for covering the sections after staining is the Krylon Plastic Spray, Crystal Clear Spray Coating No. 1302 from Krylon, Inc., Norristown, Pa.*

* Excellent also is DUCO, Dupont's Spray Magic # 438, clear.

Fig. 3.—Commercial film base.

The reel carrying the sections on film through the staining solutions is the Nikor 35 mm. developing reel. It is made of stainless steel and can be obtained from any photographic supply house.

Neither the holder nor the separator are commercially available as yet. Their prototypes were developed and hand-made for the specific purposes which they serve. The holder consists of a 3¼×4 in. glass plate 1/16 in. thick. Two strips of thin plastic

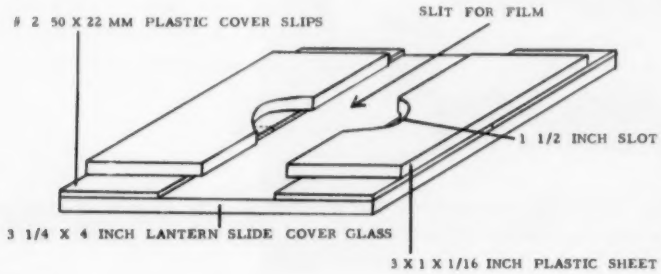


Fig. 4.—Film holder, schematic.

sheet 1/16 in. thick are glued with XAM † over two plastic cover slips on to the glass (Fig. 4). This provides a slit through which the film can be passed. In order to move high-power objectives with little clearance to the very edge of the section, two opposing semicircular sections are cut out of the two plastic sheets which make up the frame for the film on top of the glass plate. Another very simple holder of stainless steel can be made small enough to fit any of the conventional mechanical stages (Fig. 1). The separator can be glued together from strips of plastic.

Procedure

1. Strips of film of any desired length are cut from the stock roll (Fig. 3) and cleaned by dipping into acid alcohol, like conventional glass slides. They are dried with a soft cloth. Gauze should be avoided.

2. The surface of the film is coated with a thin layer of egg albumen while the film is spread out on a clean surface.

3. Paraffin sections 5μ to 10μ thick are cut, and a ribbon is placed on the water bath. The film is maneuvered under the ribbon, and the sections are picked up. When each succeeding section is from a different block, as in routine autopsy material or surgical sections, several blocks are cut in succession, and the sections are allowed to accumulate on the water surface between the separator (Fig. 2). Then, several sections are picked up at one time. After the film has been covered to capacity, it is labeled with a diamond pencil and placed in an oven of 56+ C. As with glass slides, previous short exposure to hot water from under the film will make the sections flatten out.

4. After drying, the film is loaded into 35 mm. film-developing reels (Fig. 1). Three strips each 18 in. long can be accommodated in one reel. The sections on film are stained in the usual way. An alternate method, used also with glass slides, guards against loosening of the sections. The sections are deparaffinized with xylene, placed in absolute alcohol one to two minutes, then in 0.5% celloidin five minutes, and air-dried three to four minutes. Thereafter, they are put in 80% alcohol for five minutes, then washed and stained, using the same times as those required for glass slides. The sections are dehydrated through 95% alcohol and through two changes of a mixture of one-third chloroform and two-thirds absolute alcohol, which keeps the celloidin from dissolving. Xylene is used for clearing. For differentiation the film may be pulled partly out of the reel for microscopic observation.

5. From the xylene one strip of film is removed at a time and placed face up on a blotter. It must not be allowed to dry. The film must lie flat because buckling will result in an uneven plastic coat. Straight pieces of wood or metal as weights may conveniently be placed along the perforated edges of the film. The plastic spray is applied quickly from one end to the other two to three times, until the surface appears smooth. The can with the plastic should be held approximately 6 in. above the film. The plastic is allowed to harden for 5 to 10 minutes. The spray is repeated two to three times and left to dry. The strips may be ready for microscopic examination within 10 minutes. A uniform and even spread may be obtained by placing the film strips on the inside of an open revolving drum. While the drum is spinning rapidly, the spray is applied and left to dry. If necessary, the plastic coat may be removed quickly by dipping into xylene.

6. The dry film is labeled with India ink or paper labels. Regular glass ink flakes off.

7. The sections are now ready to be studied under the microscope. A holder (Figs. 1 and 4) is essential for microscopy. It keeps the sections flat and facilitates advancing them.

8. The sections may be filed in special cabinets, stapled to the record, suspended from racks, filed individually after cutting a film strip apart, or otherwise (Fig. 5). Be-

† XAM, George T. Gurr, London, S.H. 6, England.

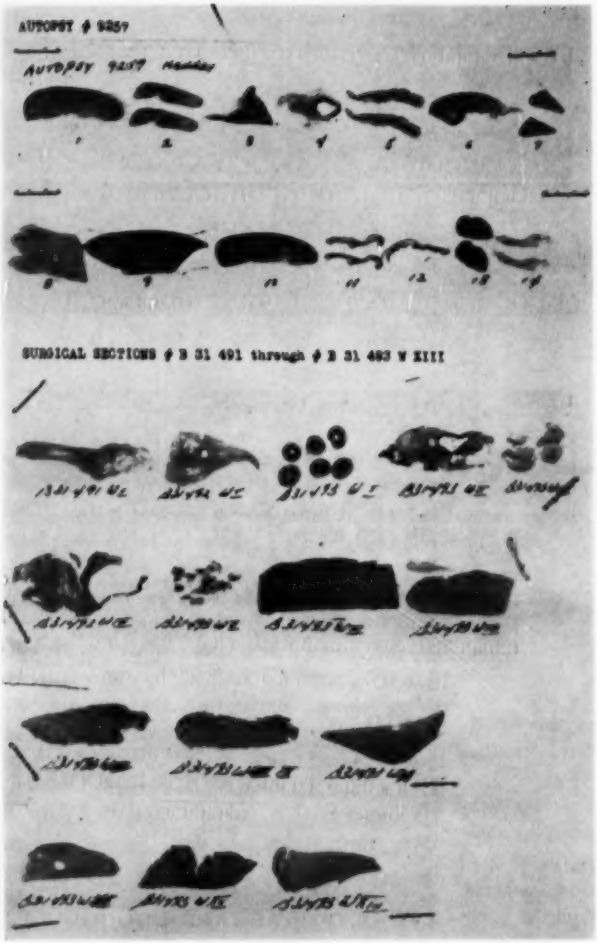


Fig. 5.—Filing. Staples should fit into perforations.

fore filing, the strips should be well dried for approximately three weeks.

Comment

Cellophane as a mounting base was used by Rasmussen as far back as 1932. In 1944 Rasmussen reported a semiautomatic assembly, using film as a mounting base for Marchi-treated sections of brain tissue.[10] Bush, in 1952, reported a successful and fully automatic device for cutting serial sections on film.[2] These methods did not become popular, because they were especially adapted for serial sections only, and because of the lack of a resistant film base. In 1955 Bush [2a] reported the use of DuPont's Mylar for tissue sections, this film being resistant to solvents.

During the past 20 years many articles have appeared advocating the use of plastic cover slips or plastic sprays instead of glass cover slips.[1,3,5,8,9,12] An extensive review of various mounting media, and their properties was reported in 1953 by Lillie.[6] Additional attempts at taking advantage of the flexibility and low cost of various film materials in the preparation of histologic sections were reported by Minckler,[6a] and Bush and Hewitt.[2b]

The potentialities of 35 mm. film as a mounting material for histologic sections are immediately apparent. However, if the film is to compete successfully with the conventional glass-slide method, several requirements have to be met. First, the film must be inert to chemicals commonly in use during staining procedures. The Cronar P40B of DuPont fills this requirement.[11] Second, the conventional glass cover slip must be replaced by a material which matches the flexibility of the film, fixes the sections permanently on the film, displays optical properties permitting the use of conventional microscope objectives, and protects the sections from damage by drying, fading of colors, scratching, or other physical forces. Although the use of another length of the same or a thinner film over a layer of mounting fluid on top of the sections is feasible, the advantages of a hardening plastic spray are clearly manifest. Several satisfactory plastic sprays are available commercially. Finally, an alternate mounting system, as is proposed here, should have optical properties comparable to the conventional method employing glass slides and glass cover slips. The method under discussion is inferior on this count in some respects. Compelling reasons for this are the irregular surface and the varying thickness of the plastic coat, as compared with the flat surface and constant thickness of glass cover slips. While the examination of sections on film with the scanning, the low-power, or the oil ‡-immersion lens is not impeded at all, for high-power observation a high-dry lens having an adjustment collar correcting for cover slips of different thicknesses is very desirable. Still better is the use of a so-called epiobjective, such as the Wild Epi-Achromate 40 and 20.[7] Other less elaborate nonimmersion lenses of high aperture may be used successfully by manipulating the substage diaphragm or by placing a cover glass over a drop of oil on the film for the time of observation. Both the oil and the cover slip may be wiped off later without damage to the film. Figures 6, 7, 8, and 9 represent photomicrographs taken with the same high-power nonimmersion lens. The lens used has an adjustment collar with which corrections can be made for cover glasses of different thicknesses. All prints were made on identical paper (Velox No. 2). Figures 6, 7, and 8 represent one section, and the section of Figure 9 is immediately adjacent. All photomicrographs have been chosen as

‡ Cedar oil dissolves the plastic spray. Nondrying oil must be used.

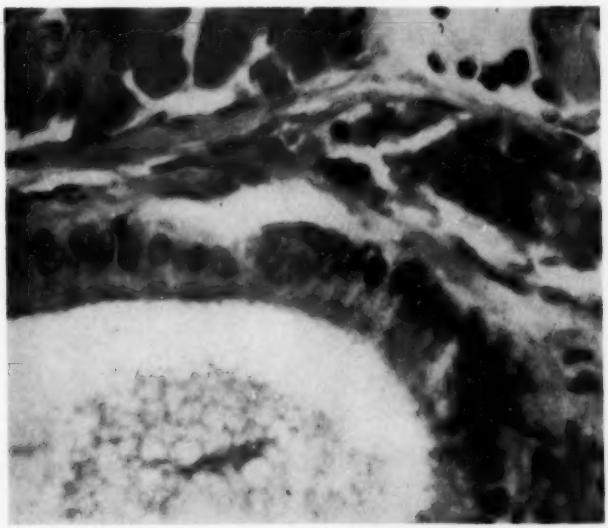


Fig. 6.—Section on film with plastic coat as cover. × 658.

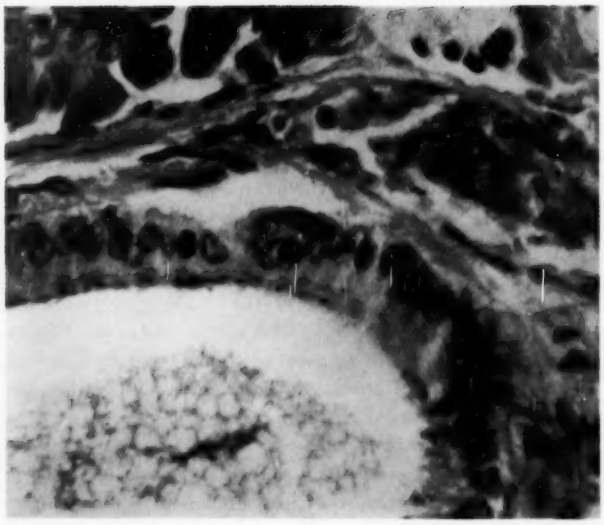


Fig. 7.—Glass cover slip over a drop of immersion oil on section on film with plastic coat. × 658.

the best representations of many takes, and after optimal manipulations of the adjustment collar of the objective, and the condensers and the diaphragms of the optical system of the photomicrographic equipment. Figure 6 is a section on film held in a holder (Fig. 2, below center) and covered only with the plastic spray. Figure 7 shows the identical section after a cover glass has been placed on top of the plastic cover over a drop of nondrying immersion oil.§ Prior to taking the photomicrograph of Figure 8, the section represented by Figures 6 and 7 was cut out of the film strip with scissors and mounted *in toto* on a glass slide with a glass cover slip. Figure 9 shows an adjacent section mounted in the conventional manner on glass.

§ Cargille's Cardinal Products, Inc., Durham, N.C., Type A.

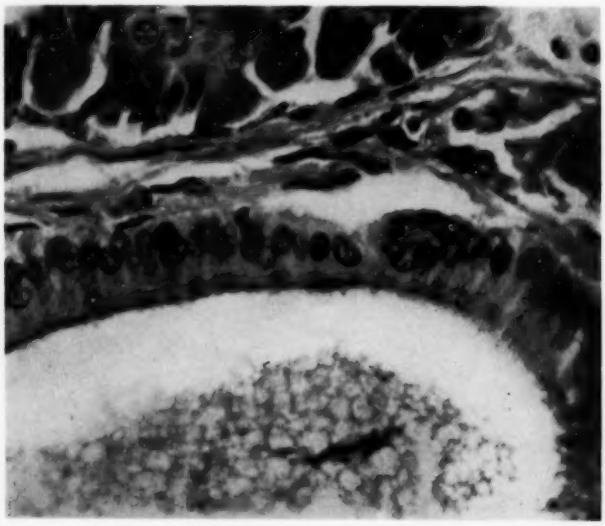


Fig. 8.—Section on film with plastic coat mounted on glass with glass cover slip. × 658.

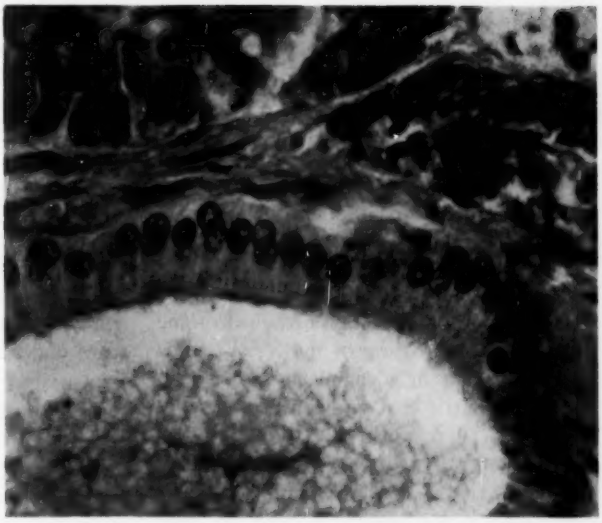


Fig. 9.—Section on glass with glass cover slip. × 658.

The most prominent advantage of the film method is the saving of both time and money. A good technician can mount approximately three sections per minute on glass with cover slips. Within the same time a strip of film carrying numerous sections can be coated with a plastic spray. Serial sections can be mounted with considerable speed, and their comparison under the microscope is greatly facilitated. While cutting sections from different blocks, time can also be saved. Several consecutive sections are allowed to accumulate on the water bath, preferably between the two edges of a separator (Fig. 2). The sections are picked up together. In contrast to glass slides and cover glasses, a strip of film can be cleaned very rapidly and without breakage. Little storage space is needed with the film method. The preservation of colors of various stains over a period of from one to three years has been excellent.

A few potential disadvantages should be discussed.

1. For observation with dry lenses of high-aperture, objectives either with an adjustment collar or epiobjectives ¶ for use without cover slips [9] have to be used, unless one uses a temporary glass cover slip over a drop of oil on the film (Fig. 7).

2. For critical studies additional preparations on glass slides may be desired, so that a separate file would be asked for. The same need may apply to special stains, although these can easily be put on separate pieces of film, which then may be conveniently attached to the original film strip on top of the corresponding section with special clips or staples fitting the marginal perforations. Sections may also be transferred to glass slides by the simple procedure of cutting out the desired sections and mounting them on glass slides in the conventional manner (Fig. 8).

3. When each section comes from a different block, a major concern is the prevention of confusion between the sections while they are floating on the water bath and waiting to be picked up together. The facility in picking up the sections together constitutes one of the time-saving features of the procedure. Simple separators (Fig. 2) can be made to form a channel matching the width of the film. The sections are first spread out on the water bath, and then moved over into the channel while gently pushing one of the edges below the surface

¶ Metalurgic lenses, used with transmitted light.

of the water. The sections are thus lined up in perfect order as they accumulate.

4. The method in its present form is not suitable for microprojection with the Bausch & Lomb carbon-arc projector, although the low-power lens produces a good picture. The scanning lens, however, gives only a fair image on the screen, and with the high-power lenses the film is destroyed within seconds.

5. The irregular surface of the plastic spray abolishes polarization of light to some extent, so that crossing of the Nicol prisms does not result in a completely dark field.

6. The plastic coat scratches more easily than glass, but a fresh coat may be applied at any time after dissolving the damaged one with xylene.

7. After several months' storage the plastic spray becomes brittle. It will break off the film base whenever the film is bent sharply. As the sections are actually embedded in the plastic they will thus be destroyed. If, however, the sections are stored flat or as a roll and are not grossly mishandled no damage to the plastic spray has been observed. As more experience with time will accumulate it might become advisable to dip old sections shortly in xylol and respray them before reexamination. This has never been found necessary so far. Encouraged by the complete absence of color fading as well as the excellent preservation of the plastic spray, studies are now in progress to evaluate application of the film method to cytologic screening also. There are numerous applications of this method. It is especially well suited for serial sections. For storage the sections may be directly attached to charts, reports, autopsy protocols, etc. (Fig. 5). Histologic sections of medicolegal autopsies, in some states, have to be filed with the Medical Examiner's Office. Strips of film carrying the sections can readily be attached to the reports and shipped in a simple envelope. Sections on film may be mailed for consultations in a plain letter envelope without fear of breakage. The Department of Pathology at Duke University School of Medicine is at the present time preparing a manual for staining procedures. Model sections of different stains are made on film and attached to the manual in special plastic jackets for ready reference. These films will serve as a teaching aid through direct visual comparison. Over a trial period routine autopsies and surgical sections were put on film, with excellent results (Fig. 5). Ancillary studies made in conjunction with research work of other, e.g., clinical, departments often do not warrant the occupation of valuable filing space under various headings in the department of pathology proper. In these, and in similar situations, sections on film may be stapled to the original research record, with the department concerned avoiding double filing. In medical schools or elsewhere collections of teaching materials may be mounted on strips of film and made available to the students. A simple cardboard 35 mm. slide frame will do nicely for a holder (Fig. 1).

The method is particularly suitable for surgical pathology. Often semiserial sections are desirable for determining margins or for finding malignant changes or evidence of early invasion, in doubtful lesions, as, for example, in certain nevi, cervical biopsy specimens, intestinal polyps, and lesions of the breast. The film method lends itself especially well to such studies. For some lesions, e.g., biopsy specimens of "in situ" cervical carcinoma and studies of certain nevi, multiple sections may become an a priori routine procedure. Extraordinary care must be exercised with labeling surgical sections.

The further development of the technique will require a still better plastic for covering the sections and better methods of applying the plastic evenly. The method, as it stands now, is excellent on all counts, and on many counts vastly superior to the glass slide-glass cover slip method. Its adaptation to the automatic microtome of Bush promises to be a further step forward in the direction of full automation of tissue laboratories.

Summary

A new method for mounting histologic sections is described, employing 35 mm. film as a base and a plastic spray instead of glass slides and glass cover slips.

The method excels by virtue of its low cost, as well as by its time- and storage-space-saving features. It is particularly applicable to serial sections and routine work in pathology laboratories of small and large hospitals, including surgical and autopsy studies.

The method could readily replace most of the routine histologic work now done on glass slides, particularly as regards day-to-day diagnostic pathology.

If properly adapted to the automatic microtome of Bush, the method ultimately may develop into full automation of the routine work of tissue laboratories.

Department of Pathology, Duke University School of Medicine.

REFERENCES

1. Bourdon, P.: Remplacement des lamelles couvre-objet par un vernis cellulosique dans le montage des preparations, Bull. histol. appl. physiol. et path. 18:238-240, 1941.

2. Bush, V.: Automatic Microtome, Science 115: 649-652, 1952.

2a. Bush, V.: Improved Automatic Microtome, Science 122:119, 1955.

2b. Bush, V., and Hewitt, Richard, E.: Frozen Sectioning, a New and Rapid Method, Am. J. Path. 28:863-873, 1952.

3. Goodchild, C. G.; Adkins, L. E., and Etherton, K. A.: Transparent Plastics with Glass Slide Preparations, Turtox News 26:148-151, 1948.

4. Heard, O. O.: Personal communication.

5. King, J. R.: Lucite as a Coating for Stained Slides of Plant Material, Stain Technol. 33:246-247, 1958.

6. Lillie, R. D.; Zirkle, C.; Dempsey, E. W., and Greco, J. R.: Final Report of the Committee on Histologic Mounting Media, Stain Technol. 28:57-80, 1953.

6a. Minckler, Dona: A Plastic Mount for Serial Sections, Am. J. Clin. Path., Tech. Sec. 6:49-50, 1942.

7. Morger, R., and Schenk, R.: Zur Herstellung, Untersuchung und Aufbewahrung Gefärbter Serienschnitte in Form von Lackfolien, Ztschr. wissensch. Mikr. 63:381-384, 1958.

8. Richards, O. W.; Small, J. H., and Collyer, P. W.: Microscopy with Plastic Substitutes for Cover Glasses, Stain Technol. 19:59-62, 1944.

9. Ransom, J. P.: An Acrylic Spray Used as a Substitute for Cover Glasses, Stain Technol. 28: 279-280, 1953.

10. Rasmussen, G. L.: A Cellophane Strip Especially Adapted for the Preparation and Study of Marchi-Treated Serial Sections, Anat. Rec. 98:331-338, 1944.

11. White, D. R.; Gass, C. J.; Meschter, E., and Holm, W. R.: Polyester Photographic Film Base, SMPTE J. 64:674-678, 1955.

12. Vroman, L.: Acrylic Spray as a Substitute for Cover-Slips, Am. J. Clin. Path. 23:516, 1953.

Pulmonary Hypertension and Plasma Thromboplastin-Antecedent Deficiency in Dogs

Experimental Induction by Infusion of Amniotic Fluid

WILLIAM E. JAQUES, M.D.; JAMES W. HAMPTON, M.D.; ROBERT M. BIRD, M.D.; KARL A. BOLTEN, M.D., and B. RANDOLPH, II.S., Oklahoma City

In 1927, Warden [1] produced pulmonary embolism by the injection of amniotic fluid into rabbits. Steiner and Lushbaugh [2] attributed the shock and sudden death observed in eight obstetrical cases to an infusion of amniotic fluid which was incident to difficult deliveries. To our knowledge, the full nature of the hemodynamic changes in the lesser circulation has not been documented. In addition to pulmonary embolization, several authors [3-5] have attributed clinical bleeding following delivery to infusions of amniotic fluid. Others [6-8] have studied the in vitro effects of amniotic fluid on the coagulation mechanism.

We wish to present preliminary observations on the effects of amniotic fluid infusions into dogs. Three aspects of the problem will be considered. What are the hemodynamic changes in the lesser circulation following the infusions? What are the effects on the coagulation mechanism? And, finally, what histological changes are produced?

Methods

In 19 experiments, nine mongrel dogs of both sexes were given intravenous infusions of autologous, homologous, or human amniotic fluid. Fluids were collected at term and filtered only if gross turbidity was present. Fluids which were obviously contaminated with blood were discarded. In 6 infusions fresh fluid was used, whereas in 13 the fluid was stored at 4 C for varying periods up to four weeks. The amount of fluid injected varied from 9 to 50 ml. In all experiments the fluid was given into a systemic vein as rapidly as possible.

All dogs were anesthetized with pentobarbital (Nembutal) sodium (30 mg/kg.). Pressures in the lesser circulation were measured through a No. 5 or 6 Cournand cardiac catheter and recorded graphically with a Sanborn 150 Polyviso carrier preamplifier. Recordings were taken from the pulmonary artery before the infusion of amniotic fluid and at frequent intervals thereafter. In six animals the changes in pulmonary hemodynamics were followed for 24 or more hours. Five of these animals received at least one additional infusion of amniotic fluid. The interval between repeated infusions varied from one day to four months. In three experiments, systemic and pulmonary pressures were recorded simultaneously, the systemic blood pressure being measured through a Henry needle inserted into a femoral artery. In the first six experiments, the solution perfused through the cardiac catheter was isotonic saline, containing 40 mg. of heparin per liter. It was perfused at a rate of approximately 30 ml. per hour. In the subsequent 13 experiments only isotonic saline was used in the cardiac catheter.

Twenty-milliliter samples of venous blood were drawn before and at intervals following the infusion of amniotic fluid. Siliconized needles and syringes were used in collecting these samples. Platelet counts were done by Olef's method.[9] Siliconized test tubes, 8 mm. in diameter, were employed to measure the coagulation and clot retraction times.[10] The colorimetric estimation of fibrinogen content utilized Parfentjev's reagent.[11] The plasma recalcification time was measured, and served as an indicator for circulating anticoagulants.[10] Blood samples, incubated at 37 C for 24 to 48 hours, were examined for fibrinolysis.[12] The

Submitted for publication May 12, 1959.

From the Departments of Pathology and of Medicine, University of Oklahoma School of Medicine.

Presented in abstract before the American Society of Experimental Pathology (Fed. Proc. 18: 64 and 483, 1959).

This investigation was supported in part by Research Grant H-3235 and by Graduate Training Grant 2A-5107, National Institutes of Health, U. S. Public Health Service.

Quick one-stage prothrombin time and the prothrombin consumption time were measured.[10] In eight experiments, the thromboplastin-generation test of Biggs, Douglas, and MacFarlane was run at a time when the utilization of prothrombin was impaired.[13,14]

To test the effects of the solutions which had been perfused through the cardiac catheter, four additional dogs served as controls. Two dogs were catheterized and the pulmonary hemodynamics measured. Both were given 250 ml. of isotonic saline through the catheter over a three-hour period, while one received an additional 50 ml. of saline through a systemic vein. Two other dogs were not catheterized but were infused systemically with an isotonic solution containing heparin (40 mg/L.). The rate of the first infusion was 30 ml. per hour, and of the second, 80 ml. per hour. Clotting studies were performed in all four control animals.

Results

Pressure Changes in the Lesser Circulation.—Following the infusion of amniotic fluid, pulmonary artery pressures were increased within 15 minutes of the infusion in 15 of 17 experiments. In the remaining two animals, one developed pulmonary hypertension more slowly, and the other died within a few minutes, showing fibrin thrombi without organization in the pulmonary bed. Generally, the pulmonary pressures reached a peak rapidly and began to recede during the second and third hours, but recatheterization days to months after the initial infusion showed that some degree of pulmonary hypertension remained in five of six dogs studied. Dogs receiving repetitive infusions of amniotic fluid tended to show an augmented hypertensive response in the pulmonary bed. Thus, by repeated infusions into the same animal, a sustained and stepwise elevation in the pulmonary artery pressures was induced. Dog 5 presented the usual response to repeated infusions and is illustrated in Figure 1. It is interesting to note that two months after the third infusion of amniotic fluid pulmonary hypertension appeared to be established. In three dogs the systemic and pulmonary arterial pressures were recorded simultaneously. In these animals there was no significant change in the systemic blood

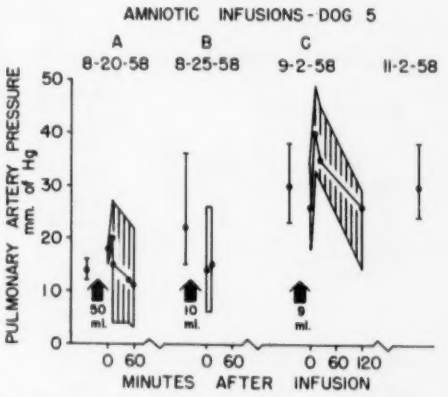


Fig. 1.—Progressive pulmonary hypertension produced by three infusions of amniotic fluid into Dog 5. These experiments are also tabulated in Table 1, Experiments 5A, 5B, and 5C. The dates of the infusions are given in the Figure. Two months following the last infusion pulmonary hypertension persisted.

pressure during the period of induced pulmonary hypertension. The infusion of saline into the two control dogs produced no significant changes in the pulmonary arterial pressure. The pertinent observations for all experiments are tabulated in Table 1. It is to be noted that the sex of the animal and the source of the amniotic fluid did not affect the pressure response. It can be seen that two dogs had elevated pulmonary pressures prior to infusion.

Changes in the Clotting Mechanism.—No clotting abnormalities were noted before the infusion of amniotic fluid. After the dogs were infused, a consistent pattern was apparent in each of four measurements of clotting. The Quick one-stage prothrombin time was unaltered with whole and dilute plasma. At the 15-minute interval, the fibrinogen content was less than preinfusion levels in 12 of 13 experiments but returned to its previous value in 24 hours. Just as promptly, there was an impaired utilization of prothrombin, as evidenced by the shortened prothrombin consumption time. This impaired utilization was marked for three hours, but always showed some improvement by the next day. The mean values of these tests are presented graphically in

TABLE 1.—*Representative Hemodynamic Changes in Dogs Following the Infusion of Amniotic Fluid* *

Experiment No., Comment	Time	Arterial Blood Pressure, Mm. Hg‡ Pulmonary	Femoral
1A †	Pre.	12/9 (11)	
15 ml. autologous fluid	2′	24/13 (18)	
	21′	32/10 (18)	
	5 hr.	27/22 (25)	
2A †	Pre.	27/8 (15)	
15 ml. homologous fluid	2′	38/15 (23)	
	1 hr.	34/11 (18)	
	4 hr. 15′	41/17 (24)	
3A †	Pre.	15/10 (12)	
20 ml. autologous and	5′	44/20 (30)	
homologous fluid	30′	22/10 (15)	
	4 hr. 30′	24/10 (15)	
	3 days	19/8 (12)	
4A	Pre.	38/17 (23)	
50 ml. homologous fluid	5′	64/34 (42)	
	45′	50/24 (32)	
	3 hr.	31/12 (20)	
4C †	Pre.	24/8 (16)	
34 ml. homologous fluid	Ad interim	41/7 (22)	
5A	Pre.	16/12 (14)	
50 ml. autologous fluid	75″	20/15 (18)	
	1 hr.	22/3 (11)	
	24 hr.	17/11 (14)	
5B	Pre.	36/15 (22)	
10 ml. autologous fluid	15″	26/5 (14)	
5C	Pre.	38/23 (30)	
9 ml. autologous fluid	14′	49/34 (40)	
	2 hr.	19/14 (16)	
	2 mo.	38/24 (30)	
6A†	Pre.	(15)	
15 ml. human fluid	5′	14/9 (12)	
7A	Pre.	25/12 (20)	
10 ml. human fluid	10′	20/11 (15)	
	15′	43/30 (32)	
	30′	34/8 (15)	
7B	Pre.	28/14 (23)	
10 ml. human fluid	1′	34/17 (24)	
	17′	45/32 (38)	
	40′	23/13 (18)	
7C	Pre.	36/20 (28)	
10 ml. human fluid	15′	24/14 (20)	
	1 hr. 15′	46/18 (30)	
7D	Pre.	28/18 (24)	150/125 (130)
10 ml. human fluid	12′	15/6 (12)	135/115 (125)
	29′	30/33 (35)	165/130 (135)
	66′	29/18 (25)	140/120 (125)
8A	Pre.	40/9 §	
20 ml. autologous fluid	1′	47/13 §	
	25′	60/16 §	
8B	Pre.	50/40 (44)	
10 ml. autologous fluid	7′	24/13 (16)	
	1 hr.	47/20 (30)	
9A	Pre.	17/10 (14)	156/152 (154)
50 ml. autologous fluid	2′	22/18 (20)	155/150 (152)
	8′	18/17 (12)	156/152 (154)
9B	Pre.	19/6 (14)	140/130 (136)
15 ml. autologous fluid	18′	(21)	165/150
11A	Pre.	30/4 (12)	
250 ml. saline	5′	24/5 (15)	
over 3 hr.	1 hr.	24/6 (16)	
	3 hr.	30/4 (15)	
13A	Pre.	34/24 (29)	
250 ml. saline	1′	36/23 (28)	
over 3 hr. +	15′	24/13 (18)	
50 ml. saline	2 hr.	31/16 (24)	

* Arabic numbers indicate individual animals, and the letters denote each infusion.
† Tissues studied histologically.
‡ Mean pressures in parentheses.
§ Right ventricular pressures.

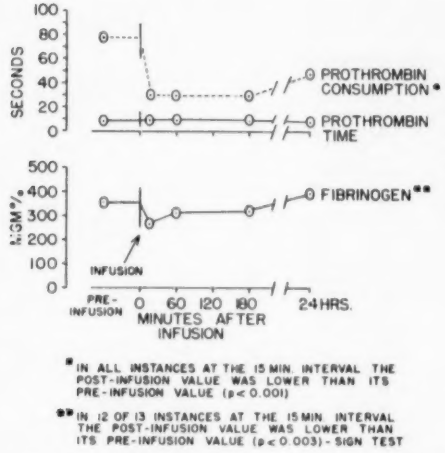


Fig. 2.—Mean values of clotting tests on dogs given 18 infusions of amniotic fluid. The Sign test was used to determine the value for P.[18]

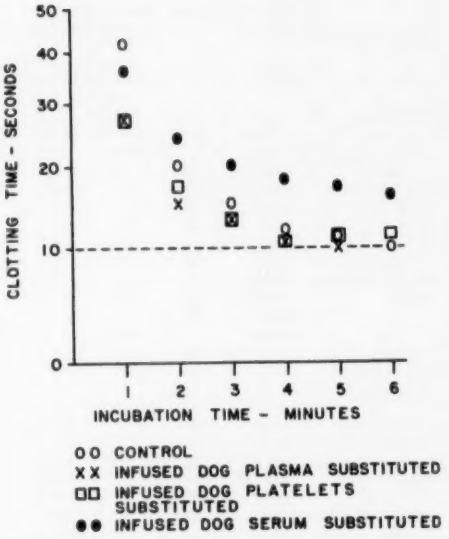


Fig. 3.—Thromboplastin-generation test, showing mean values for eight infusions. Note that in all eight instances, after six minutes of incubation, the clotting time with the infused dog's serum was longer than the control test ($P<0.008$, Sign test).[18]

Figure 2. In eight experiments, using five animals, the thromboplastin-generation test was done when an impaired utilization of prothrombin was apparent. The results were always identical. Thromboplastin generation was prolonged only when the serum factors of the infused dog were used in the reaction. Generation was normal when the experimental animal supplied either plasma or platelet factors. Figure 3 presents graphically the mean values of these eight thromboplastin-generation tests. In vitro tests with normal dog and human serum characterized the nature of the serum defect of the infused dog as being functionally similar to plasma thromboplastin-antecedent deficiency in man. The addition of normal human or normal dog $BaSO_4$ adsorbed serum or plasma in vitro corrected the serum factor deficiency. This defect was not corrected by the addition of serum from a patient who had a known plasma thromboplastin-antecedent (PTA) deficiency. The human PTA-deficient serum was corrected, however, by the addition of normal dog serum in concentrations as small as 1 in 40.

All results are enumerated in Table 2. From the tabulation it can be noted that the platelet count was always normal when prothrombin utilization was impaired, and that no evidence of a fibrinolysin was found. Neither the sex of the dog nor the source of the amniotic fluid influenced the clotting data. The results in Table 2 would indicate that the nature of the solution with which the pulmonary catheter was perfused did not influence the clotting studies. This was studied further in four control dogs described under "Methods." Only the animal which received the heparin solution at the rate of 80 ml. per hour showed an altered clotting mechanism. In this animal there was a prolongation of the Silicone coagulation time, a lengthening of the plasma recalcification time corrected by normal plasma, a shortening of the prothrombin consumption time, and an impairment in thromboplastin generation present when plasma, platelets, or serum was substituted in the reaction. On the basis of these findings, it was concluded that the clotting abnormalities induced in the experimental animals infused with amniotic fluid were not influenced by the solution perfused through the cardiac catheter.

TABLE 2.—*Clotting Studies in Dogs Following the Infusion of Amniotic Fluid*

		Measurements of Coagulation *										
										T.G.T.		
Exper. No. Comments	Time	C.T.	R.C.T.	C.R.	FIB.	LY.	PLAT.	P.T.	P.C.	4	5	6
1A †	Pre.	16	75		432	0	0.57	7	61			
15 ml. autologous	15′	24			371		0.79	11	42			
fluid	60′	26			340	0		11	45			
	180′	66	120		334		0.51	10	55			
	24 hr.	10	150		479	0	0.53	7	68			
2A †	Pre.	24	90		351	0	0.57	7	52			
15 ml. homologous	15 min.	15			371	0	0.91	13	32			
fluid	60 min.	19	110		410	0		9	43			
	180′	22			410		0.61	7	48			
	24 hr.		65		410	0			60			
3A †	Pre.	23		+++	585			10	90			
20 ml. autol. and	15′	26	74	+		0		7	34			
homol. fluid	60′	15		+	499	0		10	19			
	180′	14	46	+	549	0		10	17			
	24 hr.	16	70	+++	627	0		9	25			
4A	Pre.	15		+++	391	0		7	98			
50 ml. homologous	15′	24		+++	302	0		7	30			
fluid	60′	19		+++	273	0	0.11	9	24			
	180′	27	80	+++	312	0	1.84	9	19	13/12	13/11	13/9
	24 hr.	19		+++	351	0	1.24	5	34			
4B	Pre.	10		+++	332	0	1.77	7	29			
10 ml. homologous	180′	14		+++	253	0	2.20	8	24			
fluid	24 hr.	26		++	332			7	50			
4C †	Pre.	15	60	+++	312	0	0.98	9	120			
34 ml. homologous	15′	67	63	+	233	0	1.60	8	8	16/13	15/13	14/10
fluid					Animal Died 20 Minutes After Infusion							
5A	Pre.	16		++++	703	0	0.55	9	110			
50 ml. autologous	15′	21		+++	627	0	1.08	10	90			
fluid	60′	17		++++	549	0	1.42	10	65			
	180′	8		++++	549	0	1.26	10	75			
	24 hr.	18		++++	627	0	1.43	10	110			
5B	Pre.	19		+++	411	0	7.74	10	125			
10 ml. autologous	15′	12		++++	351	0	11.9	9	55			
fluid	60′	17		++++	302	0		7	41			
	180′	27		+++	253	0		6	24	18/11	17/9	15/8
	24 hr.	22		++++	449	0	1.35	8	36			
5C	Pre.	17		+++	332	0	0.61	9	120			
9 ml. autologous	15′	20			293	0	1.01	6	45			
fluid	180′	13			273	0	0.99	6	31			
	24 hr.	8		+++	273	0	1.00	6	25			
6A †	Pre.	16		+++	194	0		9	40			
15 ml. human	15′	32		None	49	0		8	12	21/13	17/11	15/10
fluid					Animal Died Seventeen Minutes After Infusion							
7A	Pre.	23		+++	391	0		9	32			
10 ml. human	15′	10		+++	253	0	0.31	5	9			
fluid	24 hr.	4		+++	253	0	1.63	7	20			
7B	Pre.	17		++	273		0.26	7	28			
10 ml. human	15′	26	78	None	106		0.03	8	6			
fluid	60′	30	78	None	106	0	0.15	8	6	22/15	22/12	22/10
	180′		81		194	0	1.24	7	17			
	24 hr.		70		351		0.30	6	15			
7C	Pre.	18	85	Poor	152	0	0.49	7	20			
10 ml. human	15′	74	95	Poor	115	0	0.34	7	8			
fluid	60′	17	90	None	152	0	0.80	8	12			
	24 hr.	19	104		233		0.16	6	15			
7D	Pre.	11	117	++++	322	0	0.10	7	19			
10 ml. human	15′		120	++++	273	0	0.17	7	11			
fluid	60′	17	120	++++	322	0	0.18	7	10	13/10	13/10	13/12
8A	Pre.	6	75	++++	332	0	1.45	8	110			
20 ml. autologous	15′	10	65	++++	233	0	0.32	8	23			
fluid	60′	11	75	Poor	233	0	0.22	6	40			
	24 hr.	10	78		480	0	0.50	8	35			
9A	Pre.	8	105	++++	431	0		7	115			
50 ml. autologous	15′	10	95	++++		0		6	40			
fluid	60′	11	98	++++	352	0		7	25			
	24 hr.	10	110	++++	313		2.72	7	75			

9B	Pre.	7	92		175			7	92	
20 ml. autologous	15′	5	90		175			7	15	
fluid	180′	14	100		115		1.38	7	11	15/10 15/11 16/14
	24 hr.								100	
9C	Pre.	14	105	++++	214		0.87	8	82	
15 ml. autologous										
fluid	60′	16	98	+++	200		0.84	7	14	18/11 17/10 16/11
11A	Pre.	12	120		607	0		7	95	
250 ml. saline	15′	12	125		587	0	0.40	7	85	
over 3 hr.	60′	12	110		587	0		7	90	
	180′	11	115		598	0	0.59	7	80	
12A	Pre.	24		+		0			47	
Heparin (40 mg/L.)	180′	27		+++		0			39	
30 ml. per hr.										
13A	Pre.	7	70	+	234	0	1.10	7	35	
250 ml. saline	15′	11	85	+	244	0	1.38	7	29	
over 3 hr. plus	60′	9	75	+	234	0	0.90	7	30	
50 ml. saline	180′	10	80	Poor	234	0	0.78	7	29	
14A	Pre.	15	70	+++	263		1.23	7	35	
Heparin (40 mg/L.)	15′	29	120		263		1.16	7	16	
80 ml. per hr.	180′	33	640+	None	293	0	0.92	4	14	See text

* Exper. No. indicates dogs by Arabic numbers; letters refer to single or repetitive infusions. Time refers to sampling; e.g., Pre.= prior to infusion, and numerals indicate minutes after infusion. C.T.=Silicone tube clotting time, in minutes; R.C.T., plasma recalcification clotting time, in seconds; C.R., clot retraction, with ++++ indicating complete retraction in one hour and +, complete in four hours; FIB., fibrinogen, in milligrams per cent; LY., fibrinolytic activity; PLAT., platelets, 10^4/cu. mm.; P.T., one-stage prothrombin time, in seconds; P.C., prothrombin consumption time, in seconds; T.G.T., thromboplastin generation test, for serum of infused dog compared with normal serum, as substituted in the clotting reactions after incubation for four, five, and six minutes. These clotting times are in seconds.

† Tissues studied histologically.

Changes in Morphology.—Five dogs, Nos. 1, 2, 3, 4, and 6 (three females and two males), were autopsied. Dog 1 died 24 hours after infusion; Dog 4 and Dog 6 died almost immediately, the former after the third infusion, whereas the other two dogs were killed. In all dogs the viscera showed no gross pathological changes, and sections exhibited similar microscopic findings. Fibrin thrombi, squamae, lanugo, and particles resembling meconium were demonstrated in the smaller vessels of the pulmonary bed of all animals and in the kidney of one. Dogs surviving the initial infusion showed varying stages of thrombic organization. This was characterized initially by endothelial proliferation and ingrowth of fibroblasts, and later by the deposition of mucopolysaccharides and collagen in the thrombi. Additional changes in the pulmonary vessels

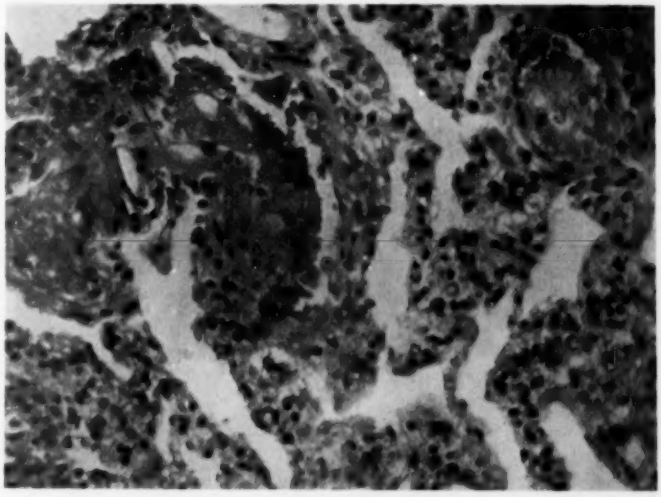


Fig. 4.—Lung. Fibrin thrombi obstruct three pulmonary arteries of a dog which died 15 minutes after infusion of amniotic fluid. Hematoxylin and eosin; reduced to 69% of mag. × 375.

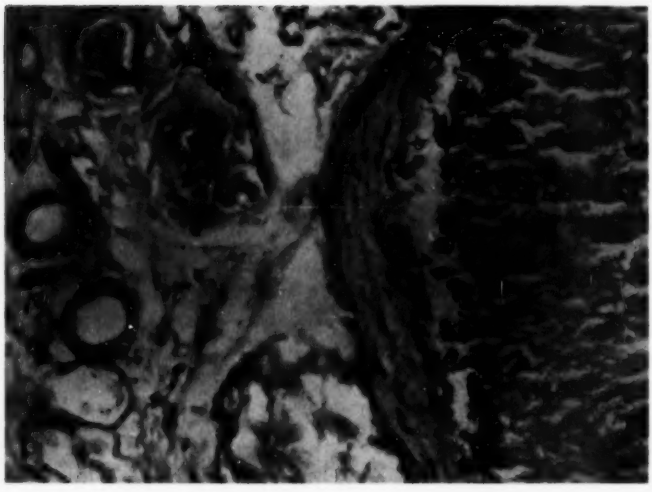


Fig. 5.—Lung. A large pulmonary artery is situated next to a major bronchus. The artery contains a thrombus which shows early organization. Hematoxylin and eosin; reduced to 69% of mag. × 375.

remote from the points of fibrin deposition were characterized by increased metachromasia of the intima and interstitial tissue of the media. Representative photomicrographs are shown in Figures 4, 5, and 6.

Comment

Since pulmonary hypertension can be produced by intravenous injections of autologous clots,[16] glass beads,[17] and other foreign substances,[18] it was not surprising that it developed after the infusion of the contents of the amniotic sac. In the experiments reported here the amounts of amniotic fluid infused did not influence critically the degree of the hypertension. We think that it is important to emphasize the rapidity with which the hypertension developed. Quantities of amniotic fluid larger than those used in our experiments probably account for the systemic hypotension previously reported.[19] The morphological changes noted in the present study are similar to those previously reported.[2] The formation of fibrin thrombi

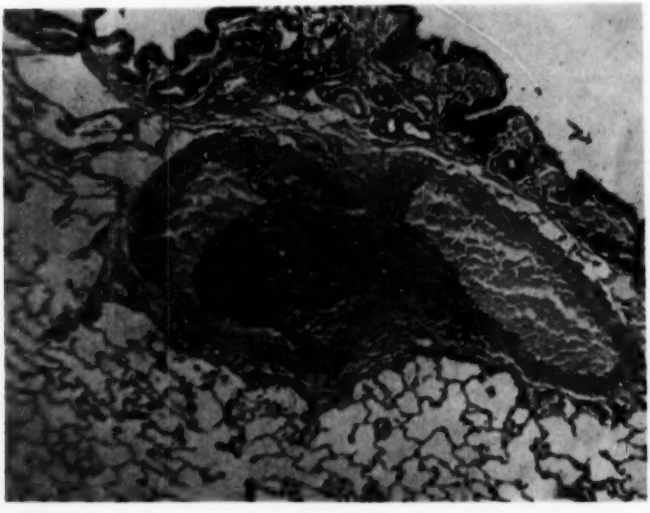


Fig. 6.—Lung. A major pulmonary artery shows a more advanced degree of organization; producing an eccentric intimal thickening. Alcian blue; reduced to 69% of mag. × 110.

undoubtedly accounts in part for the changes in pulmonary hemodynamics but does not satisfactorily explain the rapidity with which they appear. A neurogenic mechanism of pathogenesis [15] could explain these acute changes more readily than attempting to explain them on the basis of mechanical blockage.[17] Pulmonary bed pressures tended to increase progressively with repeated infusions, and in one animal it has been established that a sustained pulmonary hypertension was produced.

Two defects in the clotting mechanism were consistently noted in our experiments. The first was a fall in fibrinogen. Such a finding is in keeping with clinical reports.[4] In the dogs, no lysis was noted, and the extent of the intravascular deposition of fibrin seemed less than an adequate explanation. The second defect resulted in a deficient thromboplastin production. The thromboplastin-generation test localized this defect to the serum. The one-stage prothrombin time was normal. Adsorption with $BaSO_4$ did not alter the ability of normal plasma or serum to correct the abnormal generation of thromboplastin. Reasoning from human clotting data, it was concluded that the abnormality which the dogs developed was functionally the same as plasma thromboplastin-antecedent (PTA) deficiency in man.[20] This inference was supported by the failure of serum from a PTA-deficient human to correct the dog's thromboplastin generation. A similar serum thromboplastin deficiency has been reported to occur with the Shwartzman reaction in rabbits [21]; however, this is the first time that such an experimental model has been documented in the dog. As attractive as it is to assume that this thromboplastin-generation defect is specifically the result of the infusion of amniotic fluid, the possibility exists that the defect is a consequence of the intravascular damage produced by the fibrin thrombi.[22] If such were the case, one might resolve the dichotomy of hypercoagulability, as evidenced by fibrin thrombi, and hypocoagulability, as shown by the slowed generation of thromboplastin.

Summary

Dogs infused with autologous, homologous, and human amniotic fluid rapidly developed pulmonary hypertension without regard for the sex of the animal or the source of the fluid. The pressures in the lesser circulation increased progressively with repeated infusions.

Fibrin thrombi in varying stages of organization were demonstrated in the pulmonary vascular bed.

The animals given infusions of amniotic fluid consistently showed a prompt fall in fibrinogen and developed a defect in thromboplastin generation. This deficiency in thromboplastin generation was shown to be functionally similar to plasma thromboplastin-antecedent (PTA) deficiency in man.

University of Oklahoma, 800 Northeast 13th St. (4).

REFERENCES

1. Warden, M. R.: Amniotic Fluid as a Possible Factor in the Etiology of Eclampsia, Am. J. Obst. & Gynec. 14:292-300, 1927.

2. Steiner, P. E., and Lushbaugh, C. C.: Maternal Pulmonary Embolism by Amniotic Fluid as a Cause of Obstetric Shock and Unexpected Deaths in Obstetrics, J.A.M.A. 117:1245-1254; 1340-1344, 1941.

3. Ratnoff, O. D.; Pritchard, J. A., and Colopy, J. E.: Medical Progress: Hemorrhagic States During Pregnancy, New England J. Med. 253: 63-69; 97-102, 1955.

4. Schneider, C. L.: Etiology of Fibrinopenia: Fibrination Defibrination, Ann. New York Acad. Sc. 75:634-675, 1959.

5. Johnson, J. F.; Seegers, W. H., and Braden, R. G.: Plasma Ac-Globulin Changes in Placenta Abruptio, Am. J. Clin. Path. 22:322-326, 1952.

6. Rendelstein, F. D.; Frischauf, H., and Deutsch, E.: Über die gerinnungsbeschleunigende Wirkung des Fruchtwassers, Acta haemat. 6:18-31, 1951.

7. Schneider, C. L.: Coagulation Defects in Obstetric Shock: Meconium Embolism and Heparin; Fibrin Embolism and Defibrination, Am. J. Obst. & Gynec. 69:758-775, 1955.

8. Stefanini, M., and Turpini, R. A.: Fibrinogenopenic Accident of Pregnancy and Delivery: A Syndrome with Multiple Etiological Mechanism, Ann. New York Acad. Sc. 75:601-625, 1959.

9. Olef, I.: The Enumeration of Blood Platelets, J. Lab. & Clin. Med. 20:416-436, 1935.

10. Tocantins, L. M., Editor: The Coagulation of Blood: Methods of Study, New York, Grune & Stratton, Inc., 1955.

11. Fowell, A. H.: Turbidimetric Method of Fibrinogen Assay: Results with Coleman Junior Spectrophotometer, Am. J. Clin. Path. 25:340-342, 1955.

12. Ham, T. H., Editor: A Syllabus of Laboratory Examinations in Clinical Diagnosis: Critical Evaluation of Laboratory Procedures in Study of the Patient, Cambridge, Mass., Harvard University Press, 1950.

13. Biggs, R., and MacFarlane, R. G.: Human Blood Coagulation and Its Disorders, Ed. 2, Springfield, Ill., Charles C Thomas, Publisher, 1957.

14. Miale, J. B.: Laboratory Medicine: Hematology, St. Louis, C. V. Mosby Company, 1958.

15. Dixon, W. J., and Massey, F. J.: Introduction to Statistical Analysis, Ed. 2, New York, McGraw-Hill Book Company, Inc., 1957, Chap. 17, pp. 280-287.

16. Jaques, W. E., and Hyman, A. L.: Experimental Pulmonary Embolism in Dogs: A Study of the Physiologic and Anatomic Changes Following Repeated Injections of Autogenous Clots, A.M.A. Arch. Path. 64:487-493, 1957.

17. Williams, M. H., Jr.: Mechanical vs. Reflex Effects of Diffuse Pulmonary Embolism in Anesthetized Dogs, Circulation Res. 4:325-331, 1956.

18. Price, K. C.; Hata, D., and Smith, J. R.: Pulmonary Vasomotion Resulting from Miliary Embolism of the Lungs, Am. J. Physiol. 182:183-190, 1955.

19. Hardaway, R. M., III, and McKay, D. G.: Disseminated Intravascular Coagulation: A Cause of Shock, Ann. Surg. 149:462-470, 1959.

20. Rosenthal, R. L.; Dreskin, O. H., and Rosenthal, N.: New Hemophilia-like Disease Caused by a Deficiency of a Third Plasma Thromboplastin Factor, Proc. Soc. Exper. Biol. & Med. 82:171-174, 1953.

21. Kliman, A.; Alexander, B., and McKay, D.: Coagulation Defect Associated with the Generalized Shwartzman Reaction, Fed. Proc. 18:486, 1959.

22. Penick, G. D.; Roberts, H. R.; Webster, W. P., and Brinkhous, K. M.: Hemorrhagic States Secondary to Intravascular Clotting: An Experimental Study of Their Evolution and Prevention, A.M.A. Arch. Path. 66:708-714, 1958.

Induction of Neoplasms in Rat Thyroid Glands by Low Doses of Radioiodine

G. D. POTTER, Ph.D.; S. LINDSAY, M.D., and I. L. CHAIKOFF, M.D., Berkeley, Calif.

Several reports dealing with the induction of benign and malignant thyroid neoplasms in rats by single injections of I^{131} or by administration of x-rays have appeared.[1-5] These radiation-induced neoplasms differ both cytologically and histologically from the naturally occurring thyroid neoplasms in the rat.[1-3] The highest incidence of radiation-induced tumors in our study [1] was observed in rats that had received 10μc. or 25μc. of I^{131}. In some of the rats that received 200μc.-400μc. of I^{131} the thyroid glands were completely destroyed; in those with residual thyroid tissue the incidence of radiation-induced thyroid neoplasms was reduced, and growth of the naturally occurring thyroid tumors was inhibited. It was inferred that this residual thyroid tissue had been so damaged that it became refractory to thyrotropin stimulation.

Because of the higher incidence of induced thyroid neoplasms in rats injected with *low* doses of I^{131}, it seemed important to explore further the pathogenesis of these tumors. In the studies reported here we administered single as well as repeated low doses of I^{131}.

Experimental Method and Material

Two hundred male Long-Evans rats were used in this study. They were raised on Purina Laboratory Chow (which contains about 5μg. of iodine per gram) plus a twice-weekly feeding of lettuce. At 8 weeks of age, when the rats weighed about 150 gm., they were divided into two groups of 100 each. Each rat of one group received a single intraperitoneal injection of 25μc. of carrier-free I^{131}; each rat of the other group was given a total of four intraperitoneal injections of 10μc. of carrier-free I^{131} at one-month intervals. In this experiment, control rats were not examined because, in our previous study, we established the incidence of naturally occurring neoplasms in the thyroid glands and other tissues of 156 normal Long-Evans rats.[1]

Two years after the initial injection, 23 of the 100 rats that had been given injections of 25μc. of I^{131} and 28 of the 100 rats that had received 40μc. survived. The others died of respiratory or other diseases; because of extensive mutilation their tissues were not examined. The rats that survived for the two years were anesthetized with pentobarbital (Nembutal) and exsanguinated. The thyroid glands, along with tracheas and adjacent tissues, were excised and fixed in 10% neutral formalin. Multiple horizontal sections from each gland and attached trachea were prepared, and all sections were stained with hematoxylin and eosin.

Submitted for publication May 12, 1959.

This work was supported by a contract from the Atomic Energy Commission.

From the Departments of Physiology (Berkeley) and Pathology (School of Medicine, San Francisco), University of California.

Results

A. Gross Description

Approximately one-third of the irradiated thyroid glands was of normal size, with symmetrical lobes approximately 5 to 6 mm. long and 2 mm. wide. Another third of the irradiated thyroid glands displayed diffuse enlargement of either one or both lobes. These diffusely enlarged lobes had smooth, non-nodular surfaces.

The remaining third of the irradiated thyroid glands showed diffuse or nodular enlargement of one or both lobes (Figs. 1 and 2), and the lobes had lobulated surfaces and were pink or pale yellow-white in color. Some of these enlarged lobes adhered firmly to the adjacent muscles and trachea (Figs. 3 and 4). In two animals a tremendously enlarged thyroid lobe extended posteriorly, to adhere to the esoph-

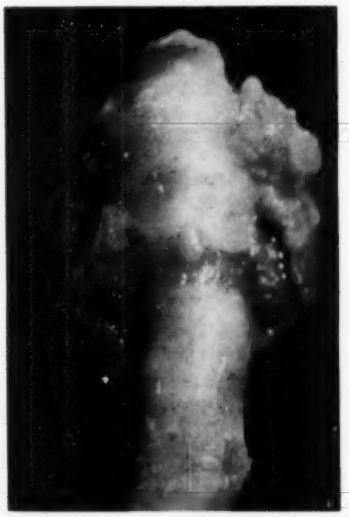


Fig. 1.—Multiple follicular adenomas of thyroid gland. The right lobe is larger, and both lobes have lobulated nodular surfaces. × 8. (10μc., × 4.) (All rats used in this study received injections of I^{131} at 8 weeks of age and were killed two years later.)

Fig. 2.—Papillary adenoma of right lobe of thyroid gland. The nodule occupies the entire lobe and is completely encapsulated. × 8. (10μc., × 4.)

Fig. 3.—Papillary carcinoma of left lobe of thyroid gland. The neoplasm is infiltrating adjacent muscle and the trachea. × 8. (10μc., × 4.)

Fig. 4.—Follicular carcinoma of right lobe of thyroid gland. The large mass has caused tracheal angulation. The left lobe is also enlarged and contains two macrofollicular adenomas. × 8. (10μc., × 4.)

agus. These lobes consisted of firm, yellow-pink or yellow-gray neoplastic tissue, which had invaded beyond the thyroid capsule.

In some thyroid lobes, usually those of normal size, one or two parathyroid glands were seen.

B. Microscopic Description

I. Thyroid Parenchyma.—The histologic patterns of the thyroid glands of rats that had received 25μc. and of those that had received four separate injections of 10μc. each were identical, and consisted of fairly uniform follicles slightly smaller than those of normal rats (Fig. 17). The follicles were composed of pleomorphic epithelial cells that contained abundant cytoplasm, sometimes granular or vacuolated, and hyperchromatic nuclei with closely packed, finely granular chromatin. These epithelial cells were devoid of mitotic figures. The colloid in the follicles was dense and often granular, and tended to be condensed centrally. Some colloid appeared partially calcified and laminated. The interstitial supporting connective tissue appeared normal, and there was no significant perifollicular fibrosis. Small focal collections of lymphocytes and plasma cells were found in the interstitial tissue of one gland. A pattern typical of that of Hashimoto disease consisting of diffuse infiltration with lymphocytes and plasma cells (Fig. 5) was observed involving approximately one half of a thyroid lobe in another animal. This inflammatory reaction isolated groups of small proliferating thyroid follicles, which were composed of fairly uniform thyroid epithelial cells with abundant eosinophilic cytoplasm. In one animal, showing widespread lesions characteristic of chronic lymphatic leukemia, considerable infiltration of the entire thyroid gland with neoplastic lymphocytes was observed. Acute necrotizing arteritis, observed by us earlier in thyroid glands of rats that received larger doses of I^{131},[1] was found in a thyroid artery of a rat that had received a single injection of 25μc.

II. Thyroid Neoplasms.—1. Naturally Occurring Alveolar Carcinoma of the Thyroid Gland: The thyroids of 29% and 35% of the I^{131}-injected rats (Table) contained alveolar or lobular carcinomas identical with those previously described in *normal* Long-Evans rats.[1-3] Most of these carcinomas were small, and consisted of neoplastic epithelial cells, which filled single follicles or small groups of contiguous follicles. The cells were uniform and contained few mi-

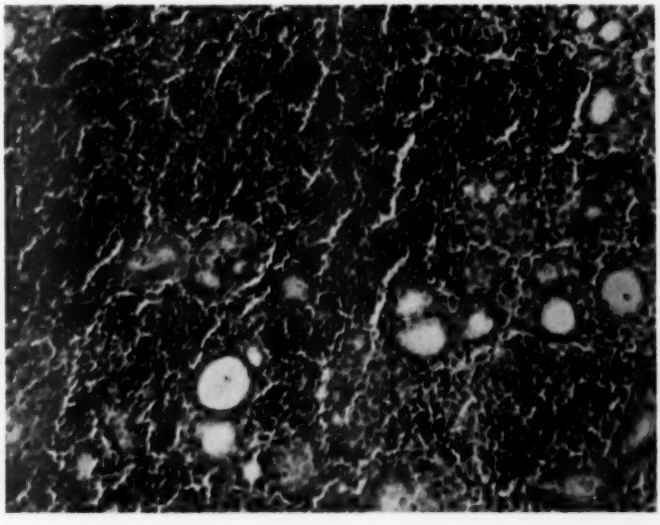


Fig. 5.—Hashimoto thyroiditis involving approximately half of one lobe. Some of the microfollicles are composed of thyroid epithelial cells with oxyphilic cytoplasm (Hürthle or Askanazy cells). Hematoxylin and eosin; reduced to 88% of mag. × 120. (10μc., × 4).

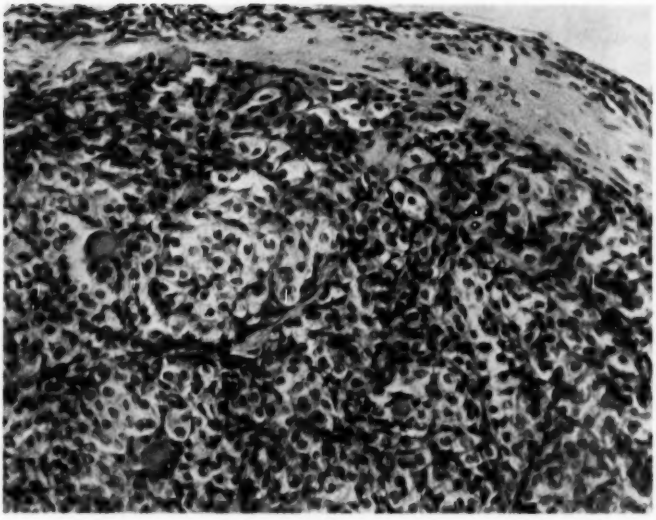


Fig. 6.—Alveolar carcinoma of thyroid gland. This naturally occurring thyroid carcinoma consists of uniform thyroid epithelial cells, some of which form microfollicles. Note limitation of the neoplasm by the thyroid capsule. Hematoxylin and eosin; reduced to 88% of mag. ×250. (25μc.)

toses (Fig. 6). In a few glands these lesions were larger, involving one or more lobules of a lobe, and, in two instances, an entire thyroid lobe. In one rat bilateral multicentric lesions of this type were found. The larger lesions replaced the thyroid parenchyma by follicular invasion, and this gave the neoplastic cells an alveolar or lobular pattern. These naturally occurring thyroid carcinomas tended to be circumscribed and, when large, were limited in growth by the thyroid capsule (Fig. 6).

2. I^{131}-Induced Thyroid Neoplasms: (*a*) Follicular adenomas in the thyroid glands of I^{131}-injected rats.

The thyroid glands of all but two of the I^{131}-injected animals contained benign thyroid nodules or adenomas identical with those described in an earlier report.[1] (The two thyroid glands in which such adenomas were not found were extensively invaded by malignant thyroid neoplasms, which undoubtedly obscured any such preexisting benign thyroid nodules.) Single or, more frequently, multiple adenomas of this type were found in one or both lobes. These multiple benign nodules were well circumscribed and were separated from the residual thyroid parenchyma by thin, intact fibrous capsules.

In the earliest stage these lesions consisted of a group of enlarged thyroid follicles lined by flattened epithelial cells and filled with colloid. These lesions were classified as early macrofollicular adenomas.

The larger nodules consisted of more numerous follicles with both microfollicular or macrofollicular patterns. Some nodules were composed mainly of greatly enlarged follicles with considerable papillary infolding of the lining epithelium. These nodules tended to compress the remaining thyroid parenchyma, and in many instances completely occupied one or both lobes. Evidences of old and recent hemorrhages were frequently observed, especially in the larger follicles of the macrofollicular adenomas. These benign thyroid nodules were composed of fairly uniform, cuboidal epithelial cells which showed only occasional mitotic figures. In the present study benign nodules displaying an extensive trabecular pattern were not observed, although this pattern was not uncommon in portions of some of the malignant thyroid epithelial neoplasms observed in these rats.

(*b*) Papillary adenomas in the thyroid glands of I^{131}-injected rats.

The thyroid glands of three of the I^{131}-injected rats contained benign papillary

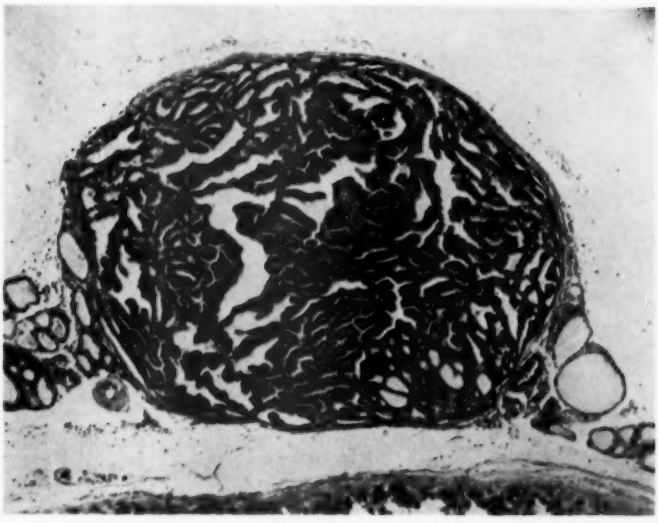


Fig. 7.—Papillary adenoma in thyroid isthmus. The pattern is almost entirely papillary. The nodule is completely encapsulated. Hematoxylin and eosin; reduced to 88% of mag. ×60. (10μc., ×4.)

adenomas. In one animal, the adenoma lay in the center of the isthmus (Fig. 7), and in the other two it was found in a lateral lobe. Unlike the malignant papillary lesions, described below, these adenomas were well circumscribed and encapsulated, with no evidence of granular or vascular invasion.

The nodules in two rats consisted of small cells with hyperchromatic, uniform nuclei and sparse cytoplasm. These nodules had purely papillary patterns, with cells lying on the surfaces of delicate, frond-like processes.

In the adenoma of the third rat the cells were larger and pleomorphic, and contained pale vesicular nuclei with prominent nucleoli (Fig. 8). Although encapsulated, this lesion closely resembled the malignant papillary neoplasms and, like them, possessed extremely numerous mitotic figures. This adenoma showed a microfollicular pattern

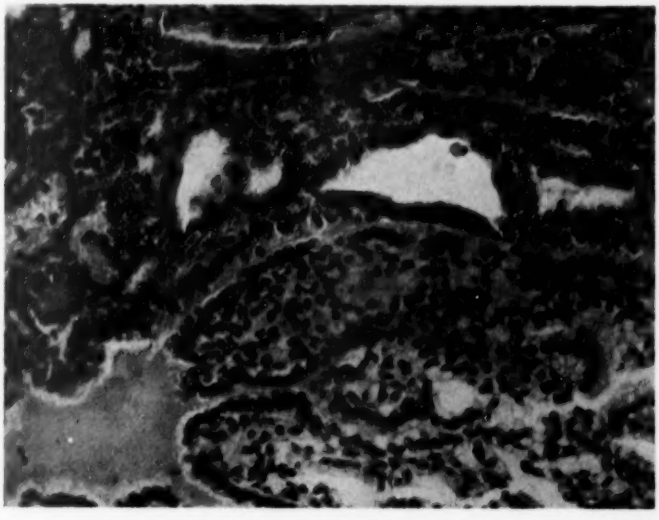


Fig. 8.—Papillary adenoma of thyroid gland. The cells vary in size. Both papillary and follicular patterns are present. Hematoxylin and eosin; reduced to 88% of mag. ×250. (10μc., ×4.)

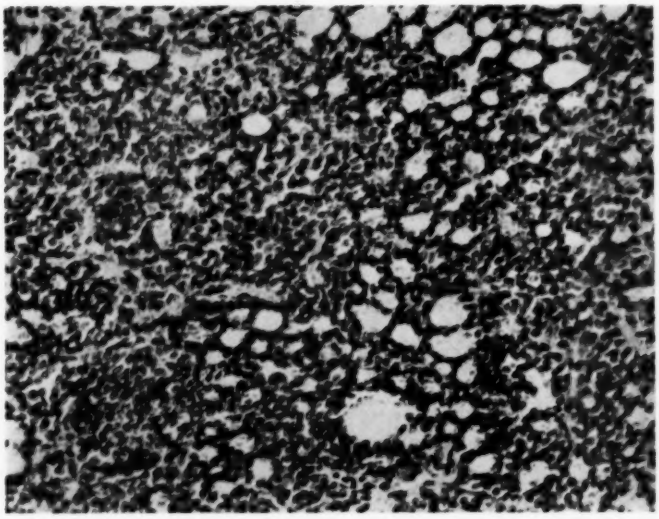


Fig. 9.—Follicular carcinoma. The pattern is microfollicular, and the follicles are devoid of colloid. Hematoxylin and eosin; reduced to 88% of mag. × 120. (10μc., × 4.)

at the periphery, and the follicles contained colloid (Fig. 8).

(*c*) Follicular and papillary carcinomas in the thyroid glands of I^{131}-injected rats.

In the present study, 6 follicular carcinomas and 6 papillary carcinomas were found in 12 of the rats that had been injected with I^{131}. These malignant thyroid epithelial neoplasms closely resembled carcinomas observed in the human being.[6]

Follicular carcinomas. The six neoplasms designated as follicular carcinomas consisted of wide sheets of neoplastic thyroid epithelial cells, some of which formed very small or normal-sized thyroid follicles (Fig. 9). In most instances the cells were small and had round, hyperchromatic nuclei and relatively sparse cytoplasm (Fig. 10). In one follicular carcinoma the nuclei were larger (Fig. 11). Moderate num-

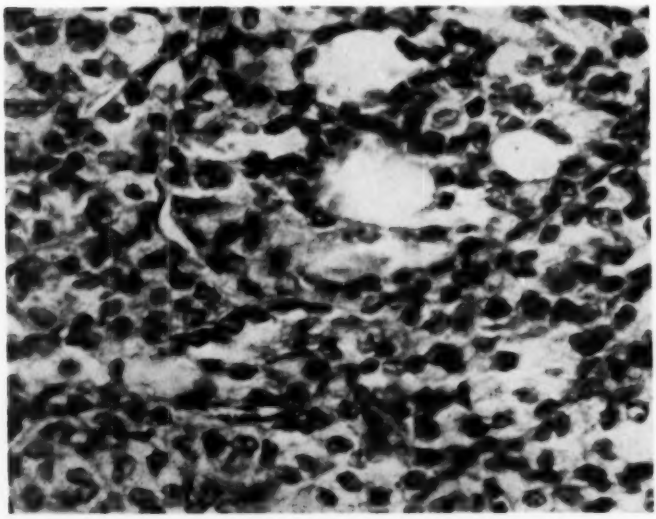


Fig. 10.—Follicular carcinoma of thyroid gland. The pattern is similar to that in Figure 9. The cells are small and have small, round, hyperchromatic nuclei. Hematoxylin and eosin; reduced to 88% of mag. × 560. (25μc.)

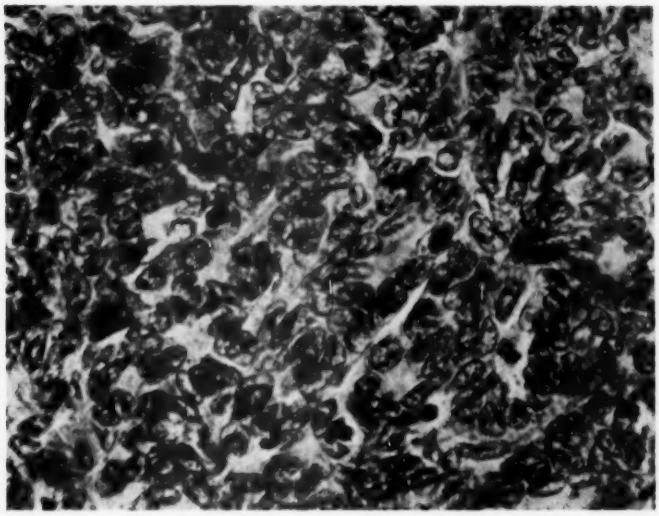


Fig. 11.—Follicular carcinoma of thyroid gland. The cells are larger than those seen in Figure 10. Poorly defined microfollicles are present. Note mitotic figures. Hematoxylin and eosin; reduced to 88% of mag. × 560. (10μc., × 4.)

bers of mitotic figures were found. The thyroid follicles formed by the neoplastic cells contained pale, vacuolated colloid. Although partly circumscribed, these lesions extended locally into the residual thyroid parenchyma, and, in one instance, through the thyroid glandular capsule (Fig. 12). In another, the main neoplasm had satellite neoplastic nodules. Two of the follicular neoplasms had invaded the lumens of the large veins.

Papillary carcinomas. The six neoplasms designated as papillary carcinomas consisted of cells larger than those seen in the follicular carcinomas. The nuclei showed vesiculation and coarse nuclear chromatin (Figs 13 and 14). Eosinophilic nucleoli were prominent in many cells. The cytoplasm was granular and vesicular and, in most instances, more abundant than that in the cells comprising the follicular carcinomas. More mitoses were found in the

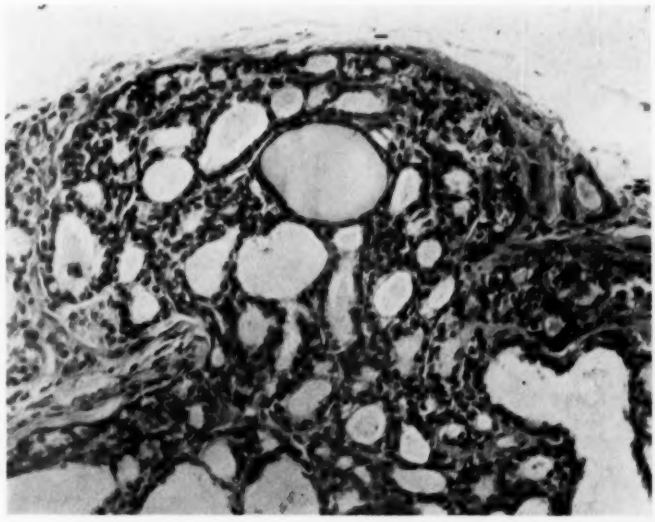


Fig. 12.—Follicular carcinoma of thyroid gland. This neoplasm was of low-grade malignancy and was mainly encapsulated. Note the follicular tissue invading through the capsule. Hematoxylin and eosin; reduced to 88% of mag. × 120. (25μc.)

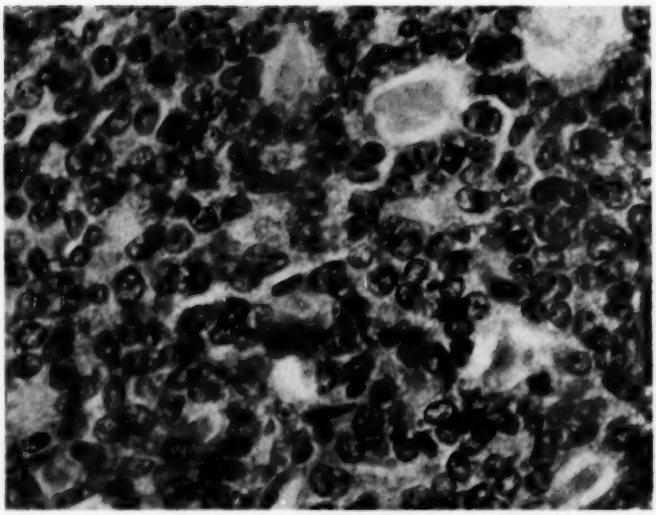


Fig. 13.—Papillary carcinoma of thyroid gland. The peripheral portion of this neoplasm has a follicular and lobular pattern. The cells and nuclei are larger and contain less chromatin than those of follicular carcinomas (Fig. 10). Hematoxylin and eosin; reduced to 88% of mag. × 560. (10μc.,)

papillary than in the follicular carcinomas. Mitoses were extremely numerous in all six papillary carcinomas, and abnormal in two of them (Fig. 15) . Only certain areas of these neoplasms showed distinct papillary structures, as a rule in the central portions of the nodules (Fig. 16). In the peripheral areas the cells had microfollicular (Fig. 13), trabecular (Fig. 14), or lobular patterns. The supporting connective tissue stroma was sparse and delicate. In two papillary carcinomas necrosis was extreme. Invasion of the residual thyroid parenchyma, extraglandular connective tissues, muscles, and large veins was a prominent feature, especially in two of the papillary carcinomas.

III. Parathyroid Lesions.—A few parathyroids had increased amounts of interstitial fibrous tissue, most abundant on the side next the irradiated thyroid gland. Undoubtedly this fibrosis was the result of the irradiation. In a few instances, the para-

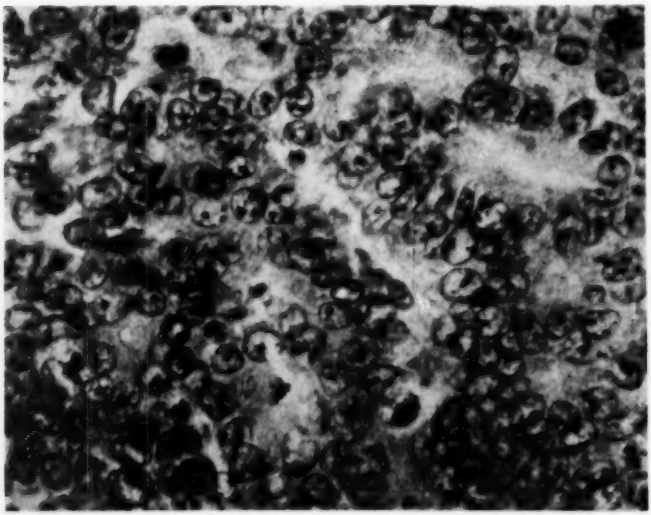


Fig. 14.—Papillary carcinoma of thyroid gland. The peripheral part of this carcinoma has a poorly defined follicular pattern. Note the large, relatively pale nuclei, characteristic of papillary carcinoma. Hematoxylin and eosin; reduced to 88% of mag. × 560. (10μc., × 4.)

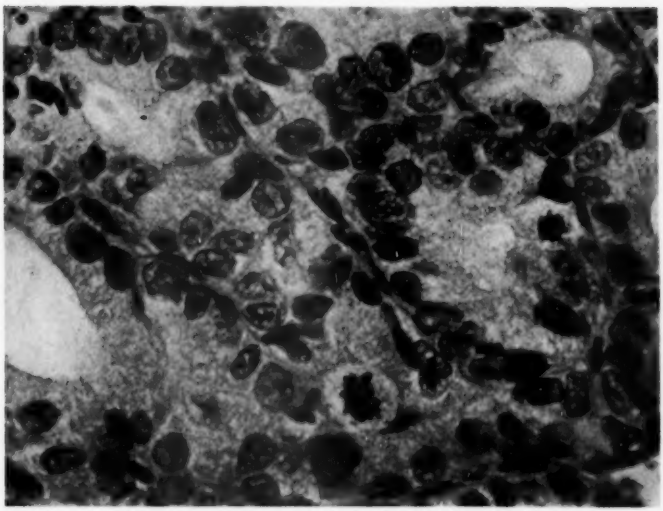


Fig. 15.—Papillary carcinoma of thyroid gland. The small follicles consist of large cells with abundant, granular cytoplasm. Note mitotic figures. Hematoxylin and eosin; reduced to 88% of mag. × 560. (10μc., × 4.)

thyroid glands were compressed by adjacent benign or malignant neoplasms in the thyroid glands.

Hyperplasia of the parathyroid glands was frequently encountered in the I^{131}-injected rats (Table). This hyperplasia was characterized by diffuse enlargement of the gland, which resulted from an increase in the number of normal-appearing parathyroid cells. In some instances, however, hyperplasia had occurred in multiple small foci (Fig. 17) in which the cells were slightly larger than normal. The nuclei of these cells were approximately 1.5 times the size of those of normal parathyroid cells. Cells that contained abundant clear cytoplasm were classified as water-clear cells.

Adenomas were also found in the parathyroid glands of the I^{131}-injected rats. These lesions were usually solitary and, in some cases, involved as much as two-thirds

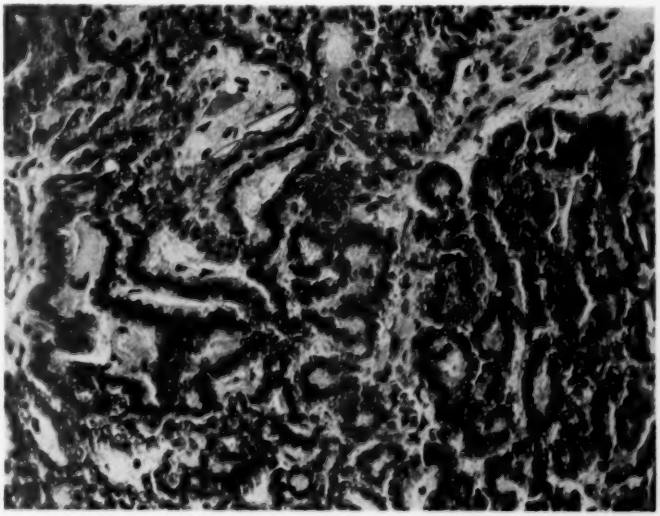


Fig. 16.—Papillary carcinoma of thyroid gland. The central portion of this neoplasm is distinctly papillary, although a few microfollicles are also present. Hematoxylin and eosin; reduced to 88% of mag. × 250. (25μc.)

Incidence of Thyroid and Parathyroid Neoplasms in I^{131}-Injected Rats

Dose of I^{131} Injected, μc.	Number Killed	Thyroid Lesions: Alveolar Carcinoma No.	Alveolar Carcinoma Per Cent	Follicular Adenoma No.	Follicular Adenoma Per Cent	Papillary Adenoma No.	Papillary Adenoma Per Cent	Papillary Carcinoma No.	Papillary Carcinoma Per Cent	Follicular Carcinoma No.	Follicular Carcinoma Per Cent	Parathyroid Lesions: Hyperplasia No.	Hyperplasia Per Cent	Adenoma No.	Adenoma Per Cent
0 *	156	47	30	1	0.6	0	0	0	0	0	0	--	--	--	--
25	23	8	35	22	96	--	--	3	13	3	13	5	22	2	9
40 †	28	8	29	27	96	3	11	3	11	3	11	3	11	7	25

* Taken from Lindsay et al.[1]

† Given as four separate injections of 10μc. each at one-month intervals.

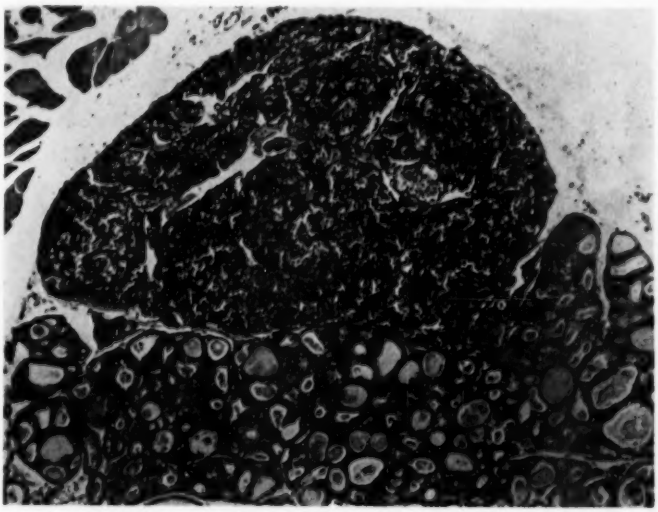


Fig. 17. — Parathyroid hyperplasia. The gland is enlarged, owing to increased numbers of normal parathyroid cells. A few proliferating foci of water-clear cells are also present. Hematoxylin and eosin; reduced to 88% of mag. ×60. (10μc., ×4.)

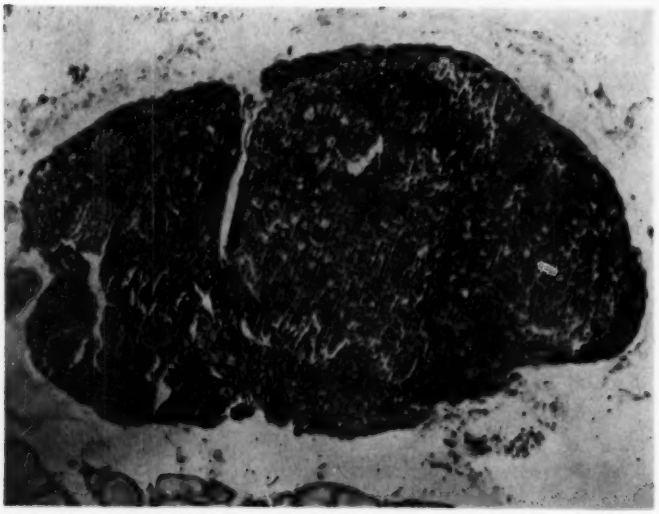


Fig. 18. — Parathyroid adenoma. The nodule on the right is circumscribed and occupies approximately two-thirds of the gland. Most of the cells of the adenoma are of the water-clear type. Note similar small proliferating foci on the left. Hematoxylin and eosin; reduced to 88% of mag. ×60. (10μc., ×4.)

of the parathyroid gland. They were discretely outlined and encapsulated (Fig. 18). Compressed margins of residual parathyroid tissue were visible at the peripheries. These adenomas consisted of parathyroid cells resembling those of the adjacent normal cells, but significantly larger. Their nuclei were approximately 1.5 times the normal size, were pale, and contained fine granular chromatin. The cytoplasm was abundant, finely granular, and eosinophilic. Some cells were even larger, and had clear, vacuolated cytoplasm. A few small follicles devoid of colloid were present in these adenomas. Mitotic figures were absent in both the hyperplastic and the adenomatous parathyroid glands. In a more recent study we have observed a high incidence of similar hyperplasias and adenomas of the parathyroid glands in normal, old Long-Evans rats.[15]

Comment

The induction of benign and malignant thyroid neoplasms in rats by the administration of low doses of I^{131} has been confirmed in this study. Previously we found that the highest incidence of both benign and malignant thyroid neoplasms occurred in rats that had received a single injection of 25μc. of I^{131}. An even higher incidence of induced thyroid neoplasms was observed in the present study. In this study, the development of malignant from benign nodules could not be demonstrated, even though this transformation was observed in the majority of papillary and follicular carcinomas described earlier.[1] However, the similarity in the patterns of the benign and malignant neoplasms suggests that the malignant lesions arose from thyroid nodules that were once benign.

Although local, regional, or distant metastases were not observed in the animals in this study, 12 of the neoplasms were designated as carcinomas because of their growth activity and their invasion of residual thyroid parenchyma, extrathyroidal tissues, and adjacent large veins.

The patterns of the benign and malignant thyroid neoplasms found in rats injected with I^{131} or exposed to x-radiation [1-3] resemble those observed in rats fed goitrogens or iodine-deficient diets.[7-13] It seems likely that, in rats so treated, focal areas of proliferation in the thyroid gland, either benign or malignant, may have resulted from prolonged stimulation by thyrotropin brought on by thyroid-hormone deficiency. The role of radiation as a primary or a contributing factor in the induction of these neoplasms cannot be decided at this time.

A high incidence of naturally occurring thyroid neoplasms, which we have designated as alveolar or lobular carcinomas, has been observed in the Long-Evans rat.[1-3] In our earlier study, about 30% of control animals showed this form of thyroid carcinoma, and the incidence of alveolar carcinomas was about 35% in the rats that had received injections of 10μc. or 25μc. of I^{131}.[1] In the present study, alveolar carcinomas were found in 29% of the rats that received a single injection of 25μc. of I^{131} and in 35% of those that received four injections of 10μc. each of I^{131}. Thus, it would appear that *low* doses of I^{131} did not influence the incidence of naturally occurring alveolar carcinomas in our rats. It should be noted here that Frantz and her co-workers [3] found an incidence of 23% in control Long-Evans rats and an even higher incidence (44%) in rats with x-irradiated thyroid glands. These authors classified the neoplastic lesions as solid carcinomas. Such naturally occurring carcinomas have little or no resemblance to those induced by irradiation (I^{131} or x-ray) or goitrogens. Their multicentric character, lobular patterns, cellular uniformity with few mitoses, and the absence of invasion beyond the gland indicate a lack of relationship between these low-grade neoplasms and those induced by I^{131}.

Thyroid neoplasms similar to the naturally occurring alveolar or lobular carcinomas observed here and elsewhere [1,3] in the Long-Evans rat have been described and classified as "type-γ-nodules" by Axelrad and Le-

blond.[12,13] They have also shown that both the incidence and the size of these neoplasms increase with age, as well as with iodine deficiency.

Of considerable interest in the present study is the high incidence of hyperplastic and neoplastic lesions of the parathyroid glands. These lesions were characterized by diffuse or focal growth activity. The latter resulted in circumscribed, discrete adenomas. Since the same lesions have been found in normal rats, they cannot be ascribed to effects of I^{131}.[15] Similar hyperplastic parathyroid glands were described by Malcolm and co-workers[14] in rats treated with thiouracil and related compounds.

Summary

The development of neoplasms was studied in the thyroid glands of Long-Evans rats two years after they had received either single injections of 25μc. or four separate injections of 10μc. of I^{131}.

Papillary and follicular carcinomas were observed in 12 of the I^{131}-injected rats.

Benign thyroid nodules or adenomas were observed in all but two of the rats given I^{131}. Three of these adenomas were of the papillary type.

Naturally occurring alveolar or lobular carcinomas were found in approximately one-third of the rats.

The high incidence of hyperplastic and neoplastic parathyroid lesions observed in the I^{131}-treated rats is not considered to be an irradiation effect.

We are indebted to Mr. Hal Strong, of the Veterans Administration Hospital, Oakland, Calif., for preparation of the photographs.

University of California, Dept. of Physiology (Dr. Chaikoff).

Addendum

Since this paper was submitted for publication, Starr and associates have reported their failure to induce thyroid carcinoma in Long-Evans rats with radioiodine.[16] The majority of their rats received amounts of I^{131} that completely, or almost completely, destroyed the thyroid gland. We have pointed out elsewhere[1] that the highest incidence of benign and malignant neoplasms is induced in rats with low doses of I^{131}. We have also suggested[1] that when larger doses of I^{131} were administered, the thyroid gland was so severely injured that proliferative responses did not occur. Starr and his associates also failed to find the naturally occurring thyroid carcinomas in the Long-Evans rats which we and others (Frantz et al.[3]) have shown to occur in about one-third of old rats of this strain. It is significant that these naturally occurring thyroid carcinomas were rarely found in rats injected with high doses of I^{131}.[1]

REFERENCES

1. Lindsay, S.; Potter, G. D., and Chaikoff, I. L.: Thyroid Neoplasms in the Rat: A Comparison of Naturally Occurring and I^{131}-Induced Tumors, Cancer Res. 17:183-189, 1957.

2. Goldberg, R. C., and Chaikoff, I. L.: Development of Thyroid Neoplasms in the Rat Following a Single Injection of Radioactive Iodine, Proc. Soc. Exper. Biol. & Med. 76:563-566, 1951.

3. Frantz, V. K.; Kligerman, M. M.; Harland, W. A.; Phillips, M. E., and Quimby, E. H.: A Comparison of the Carcinogenic Effect of Internal and External Irradiation on the Thyroid Gland of the Male Long-Evans Rat, Endocrinology 61:574-581, 1957.

4. Doniach, I.: Comparison of the Carcinogenic Effect of X-Irradiation with Radioactive Iodine on the Rat's Thyroid, Brit. J. Cancer 11:67-76, 1956.

5. Doniach, I.: The Effect of Radioactive Iodine Alone and in Combination with Methylthiouracil upon Tumour Production in the Rat's Thyroid Gland, Brit. J. Cancer 7:181-202, 1953.

6. Warren, S., and Meissner, W. A.: Tumors of the Thyroid Gland, Atlas of Tumor Pathology, Sect. IV, Fasc. 14, Washington, D.C., U.S. Armed Forces Institute of Pathology, 1953.

7. Hall, W. H., and Bielschowsky, F.: The Development of Malignancy in Experimentally Induced Adenomata of the Thyroid, Brit. J. Cancer 3:534-541, 1949.

8. Bielschowsky, F.: Chronic Iodine Deficiency as a Cause of Neoplasia in Thyroid and Pituitary of Aged Rats, Brit. J. Cancer 7:203-213, 1953.

9. Money, W. L., and Rawson, R. W.: The Experimental Production of Thyroid Tumors in the Male Rat, Tr. Am. A. Study Goiter, pp. 171-178, 1947.

10. Purves, H. D., and Griesbach, W. E.: Studies on Experimental Goitre: VII. Thyroid Carcinomata in Rats Treated with Thiourea, Brit. J. Exper. Path. 27:294-297, 1946.

11. Purves, H. D., and Griesbach, W. E.: Studies on Experimental Goitre: VIII. Thyroid Tumours in Rats Treated with Thiourea, Brit. J. Exper. Path. 28:46-53, 1947.

12. Axelrad, A. A., and Leblond, C. D.: Induction of Thyroid Tumors in Rats by a Low Iodine Diet, Cancer 8:339-367, 1955.

13. Isler, H.; Leblond, C. D., and Axelrad, A. A.: Influence of Age and of Iodine Intake on the Production of Thyroid Tumors in the Rat, J. Nat. Cancer Inst. 21:1065-1081, 1958.

14. Malcolm, J.; Griesbach, W. E.; Bielschowsky, F., and Hall, W. H.: Hyperplasia of the Parathyroids Associated with Osteitis Fibrosa in Rats Treated with Thiouracil and Related Compounds, Brit. J. Exper. Path. 30:17-23, 1949.

15. Potter, G. D.; Lindsay, S.; Sheline, G. E., and Chaikoff, I. L.: Unpublished observations.

16. Field, J. B.; McCammon, C. J.; Valentine, R. J.; Bernick, S.; Orr, C., and Starr, P.: Failure of Radioiodine to Induce Thyroid Cancer in the Rat, Cancer Res. 19:870-873, 1959.

The Introduction of Fat into Thrombi

An Experimental Study

JOEL CURRAN, M.D.; WILBUR A. THOMAS, M.D., and ROBERT M. O'NEAL, M.D., St. Louis

Various hypotheses have been advanced concerning the pathogenesis of atherosclerosis, especially as regards the initiating event. Two opposing theories are prevalent today, each supported by considerable evidence. The proponents of one theory hold that arterial plaque formation is initiated by deposition of lipid beneath the endothelial lining cells.[1] The proponents of the other, opposing, theory hold that the plaque formation is initiated by the deposition of fibrin on the endothelial surface, which is incorporated into the intima.[2] The results of the study presented herein suggest a pathogenesis that is in accord with most or all established facts concerning atherosclerosis that have been presented by both the "pure lipid" and the "pure thrombotic" school. According to these observations, atherosclerotic lesions may well result from the deposition of a combination of lipids and thrombi in close association, a possibility easily compatible with the morphology of these lesions in man.

Previous experimental work has indicated some more-than-casual relationship between fibrin and fat. Recently we reported the frequent occurrence of arterial thromboses and resultant myocardial and renal infarcts in rats fed a diet containing cholesterol, thiouracil, sodium cholate, and saturated fats.[3,4] These results, as well as those of others,[5] suggest that alterations of blood lipids may actually precipitate thrombosis. In other studies performed in rabbits, the results suggested that even after clotting has occurred, certain orally administered fats interfere with fibrinolysis; intermittent fatty meals of butter or oleomargarine markedly increased the yield of thromboembolic-induced pulmonary arterial lesions (as compared with control animals given water instead of fat).[6,7]

Another aspect of the results of previous experiments with thromboembolic-induced pulmonary arteriosclerosis was of even greater interest to us; with oil red O staining of frozen sections of the lungs of these rabbits, fat was demonstrable microscopically in occasional thrombi and in some fibrous intimal lesions, even though no stainable fat was demonstrated in other structures, suggesting that blood lipids tend to concentrate within a thrombus and are still present when it has been completely organized and converted to a fibrous intimal plaque. The numbers of lesions containing fat were too small to allow comparison between the rabbits that had received weekly supplements of fats by gastric tube and the water-fed controls, but individual lesions contained more fat than we felt would likely be present from disintegration of the waxy envelopes of the few red blood cells present in the thrombi, a source of fat previously suggested by others.[8]

The present report presents results from an additional series of experiments testing the effect of daily feeding of fat on pulmonic arterial lesions, produced as in previous work by injecting thrombi intravenously into rabbits. These experiments

Submitted for publication May 25, 1959.

From the Department of Pathology, Washington University School of Medicine.

Presented before the Intersociety Session on Atherosclerosis at the meeting of the Federation of American Societies for Experimental Biology, April 16, 1959.

Supported by U.S. Public Health Service Grant H-1820 from the National Heart Institute, National Institutes of Health, Bethesda, Md., and by a grant from the Nutrition Foundation.

were designed to increase the lipid intake of the rabbits to their limit of tolerance and perhaps provide definite morphologic evidence of an affinity between fat and fibrin. We hoped to demonstrate more clearly (1) whether localization of blood lipids occurs in thrombi, and (2) whether this localization, if present, is enhanced by a high intake of dietary fat. Butter was selected as the principal source for dietary fat because it is a principal constituent of our most reliable thrombogenic diet for rats [3,4] and had been demonstrated to interfere with clot lysis in rabbits.[6,7]

Materials and Methods

Young adult New Zealand White rabbits were maintained in air-conditioned rooms and offered 100 gm. of Purina Rabbit Pellets daily and water as desired. Three groups of animals were established which received injections of clot suspension into ear veins at weekly intervals for six weeks. Additional daily (except Sunday) feedings by gastric intubation were begun on the same day as the first injection of clot and were 50 ml. of water (Group I), 25 ml. of melted butter (Group II), 1 gm. of cholesterol in 50 ml. of water (Group III), and 25 ml. of melted butter plus 1 gm. of cholesterol (Group IV), respectively. It was soon found that the survival of rabbits fed butter was poor; so three more experimental groups were established which received no tube feedings (Group V), 10 ml. of melted butter (Group VI), and 10 ml. of butter plus 0.5 gm. of cholesterol (Group VII), respectively, daily. In these latter three groups feedings were initiated one week prior to the first injection of clot.

In addition to the controls already mentioned, two small groups of six animals each were established simultaneously with Groups I, II, and III. One received 1 gm. of cholesterol (Group VIII) and the other 25 ml. of butter (Group IX) daily, and both received 10 ml. of saline intravenously once a week (Table).

Whole blood for preparation of the clot suspension was obtained by cardiac puncture from normal rabbits and, under aseptic conditions, was stirred rapidly with a metal rod until no more clot formed. It was washed briefly in isotonic saline and placed in a Waring Blendor in a volume of isotonic saline equal to that of the whole blood from which the clot had come. The Waring Blendor was operated at high speed for 5 to 10 minutes, breaking the clot into fragments that would pass readily through an 18-gauge needle. To maintain a uniform suspension, the mixture was stirred with the Waring Blendor for two to three minutes each time prior to drawing it into the syringe. Animals that survived the experiment were killed with intravenous injection of formalin one week after the last (sixth) injection of the clot suspension.

Autopsies were performed on all animals that survived for more than one week after the beginning of the experiment. The entire thoracic contents were fixed in 10% formalin. One block of tissue was taken from each upper and each lower lobe of the lungs for paraffin sections, which were stained with aldehyde fuchsin-Van Gieson-iron hematoxylin. The sections from each rabbit were treated as a unit, and the extent and severity of arterial lesions were determined by a method described in a previous publication.[8] The lesions considered included all arteriosclerotic plaques as thick as or thicker than the wall of the containing artery and all clearly recognizable organizing or organized thrombi. In addition, other blocks of lung (two or more) were taken from each rabbit

Groups of Rabbits Established in an Effort to Cause Fat Deposition in Thromboembolic-Induced Pulmonary Arterial Lesions

Group	Daily Tube Feeding	No. Survived 1 Wk.	No. Survived 7 Wk.	Fat in Lesions
I	50 ml. water *	15	10	Rare tiny droplet
II	25 ml. butter *	27	4	Some loaded with fat even at 8 days
III	1 gm. cholesterol *	25	14	Same as Group II but more in adventitial macrophages
IV	25 ml. butter plus 1 gm. cholesterol *	15	0	Most fat seen in any group
V	No feeding *	20	14	Rare lesion with fat
VI	10 ml. butter *	30	15	Some loaded with fat
VII	10 ml. butter plus 0.5 gm. cholesterol *	37	9	More of fat in adventitial macrophages
VIII	1 gm. cholesterol (no I.V. clots)	6	4	No thromboembolic lesions
IX	25 ml. butter (no I.V. clots)	6	0	No lesions
	Total	181	70	

* Received 10 ml. of clot suspension intravenously weekly for 6 weeks.

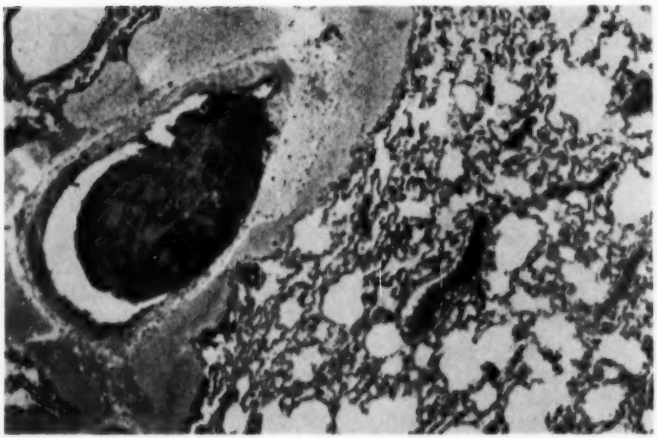


Fig. 1.—A very recent thrombus within a large pulmonary artery of a rabbit that had received 25 ml. of butter and 1 gm. of cholesterol daily for eight days. The animal died one day after an intravenous injection of blood clots. The black material within the gray thrombus is lipid. Oil red O; reduced to 69% of mag. × 120.

for frozen sectioning or polyethylene glycol embedding[8] and sectioning, and stained with oil red O for fat. The lesions thus stained and observed with light microscopy were graded in each rabbit on an arbitrary 1+ to 4+ basis for the amount of fat in the lesions proper, in the arterial walls, and in the perivascular connective tissue.

Results

Only 15% of those animals fed 25 ml. of butter daily (not considering the huge number dying in the first week) and 50% of those fed 10 ml. of butter daily survived for the duration of the experiment, as compared with 66% and 70% of the respective controls. Cholesterol feedings alone were tolerated better (56% survived). In most instances death of the butter-fed animals was apparently due to diarrhea and dehydration. In all groups occasional deaths occurred within minutes after the administration of the clot suspension, undoubtedly due to the massive pulmonary embolization.

The results of microscopic study of oil red O-stained sections are presented briefly in the Table. Stainable lipid was a prominent part of the thrombotic lesions found in the pulmonary arteries of many of the rabbits that were fed butter and/or cholesterol and given the clot suspension (Figs. 1-5), whereas fat was a rare finding in any of the lesions of the respective controls (Groups I and V), which were not tube-fed a dietary supplement of fat. On the latter an occasional single globule of fat

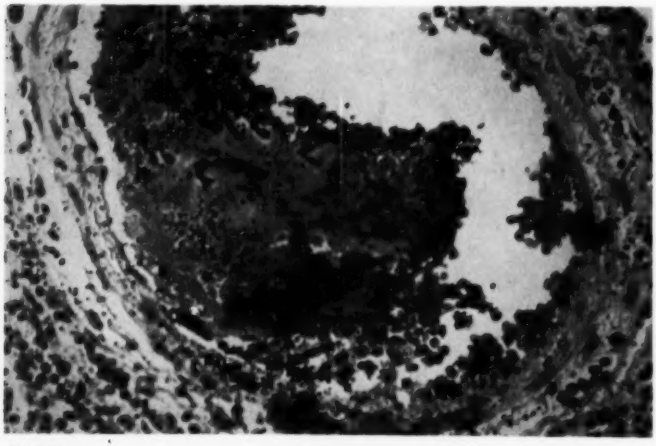


Fig. 2.—Another, smaller pulmonary artery from the same rabbit as in Figure 1. The clot in this vessel probably formed post mortem, but droplets and clumps of lipid, shown as black, are prominent throughout the material. Oil red O; reduced to 57% of mag. × 620.

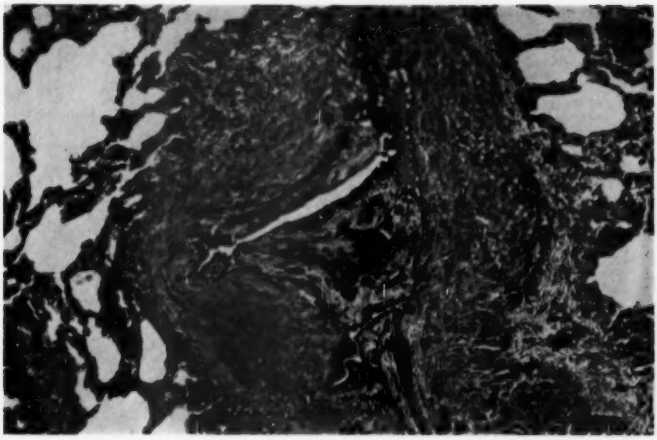


Fig. 3.—Organizing thrombus in a muscular pulmonary artery of a rabbit that had received 25 ml. of butter daily and weekly intravenous clots for six weeks. The projection of the thrombus into the arterial lumen is completely endothelized, and no remaining fibrin is seen. Oil red O; reduced to 57% of mag. × 250.

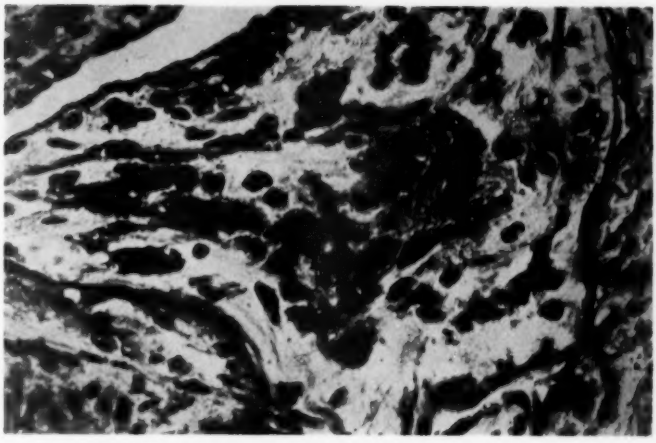


Fig. 4.—Higher magnification of the lesion in Figure 3. Fibroblasts are scattered through the loose connective tissue that has replaced thrombotic material. The fat, seen as black, lies mostly in macrophages. Oil red O; reduced to 57% of mag. × 870.

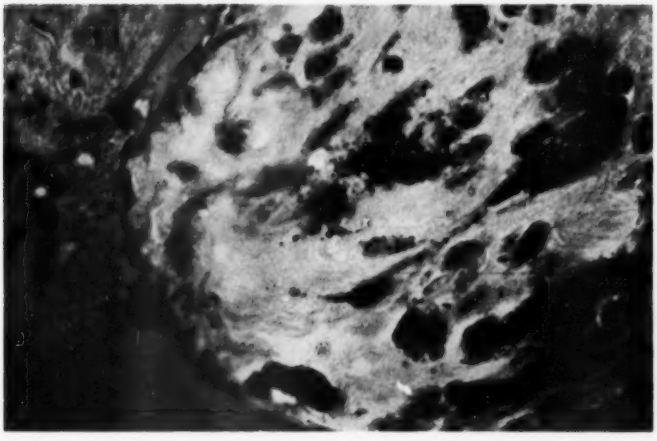


Fig. 5.—Another lesion in approximately the same stage of organization as that in Figure 4. Again, the lipid particles are principally in macrophages. Oil red O; reduced to 57% of mag. × 1,250.

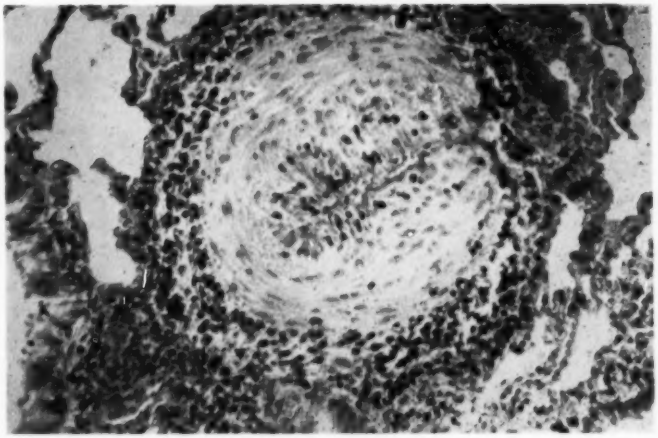


Fig. 6.—Numerous fat-filled macrophages, seen as black dots, present in the adventitia of a pulmonary artery of a rabbit fed cholesterol and given intravenous clots. Some macrophages are also present in the intima, but no thrombus is present. The presence of lipid-filled macrophages in the adventitia was a common finding in rabbits fed cholesterol. The contracted state of this artery, frequent in all experimental groups, gives the false impression that there is intimal thickening or muscular hypertrophy or both. Oil red O; reduced to 69% of mag. × 420.

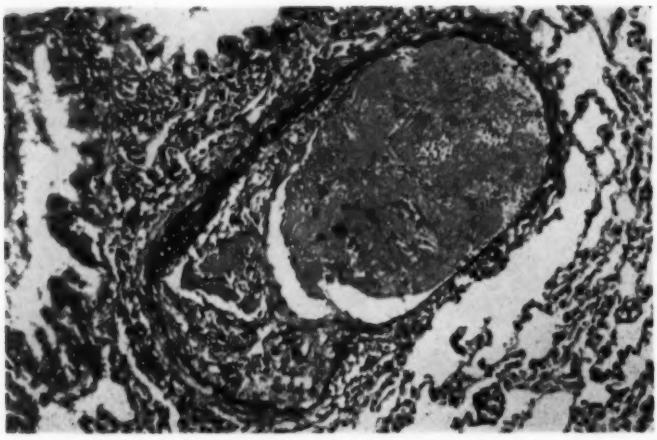


Fig. 7.—A muscular pulmonary artery containing a recent thrombus organizing at the lower left. This illustrates the manner in which fibrous intimal lesions can be produced by the organization of a thrombus. Aldehyde fuchsin-Van Gieson-hematoxylin; reduced to 69% of mag. × 280.

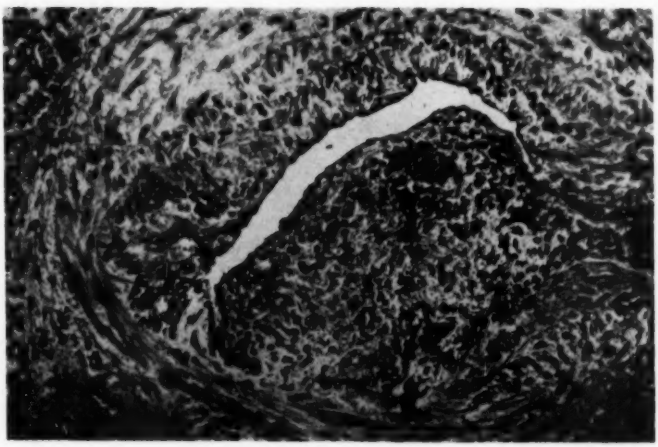


Fig. 8.—A very cellular organizing thrombus in a rabbit not receiving a dietary supplement of fat. Three globules of fat are noted within the lesion. The occurrence of lipid in the thromboembolic-induced lesions of rabbits on a diet of laboratory Chow is not common, but is regularly found in a small percentage of the lesions. Oil red O; reduced to 69% of mag. × 480.

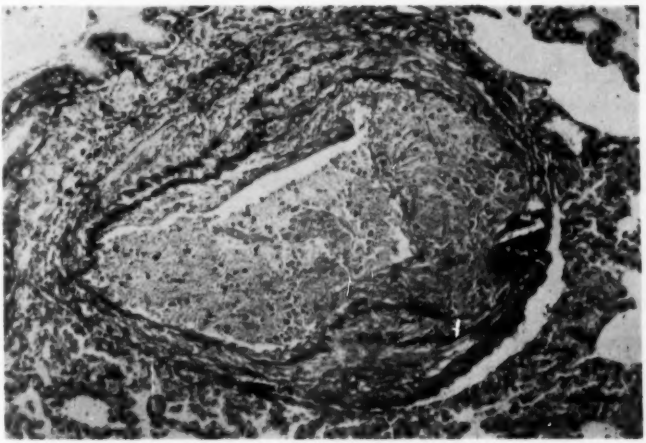


Fig. 9.—A fibrous intimal lesion characteristic of arteriosclerosis in a pulmonary artery of one of the rabbits that received intravenous injections of clot suspension. The arterial wall beneath the lesion appears thin, and the internal elastica is partially disrupted. Aldehyde fuchsin-Van Gieson-hematoxylin; reduced to 69% of mag. × 220.

was noted, in contrast to some of the lesions from the butter-fed group, where large amounts of fat were found; even some animals that died as early as the eighth or ninth day of the experiment had much fat in the thrombi. The fat in thrombi appeared to be both extracellular and within macrophages. However, the occurrence of fat in thrombi tended to be variable, with even some fat-fed animals that survived the entire six weeks, having thrombi virtually free of lipid. It was also present in the adventitia of the vessels with and without thrombi and in practically all fat-fed rabbits that survived more than a few weeks. Fat-filled macrophages were especially prominent in the adventitia of vessels in rabbits fed cholesterol (Fig. 6). In animals of all groups that received intravenous clot suspension, thrombi were found in all stages of organization, from very recent thrombi to completely organized ones (Figs. 7-10) and averaged 6 per animal in the four sections of lung examined. The experimental design was not such as to allow satisfactory comparison of numbers of lesions among the various groups, although in previous experiments of different design butter-fed rabbits had more lesions than water-fed controls.[6,7] In the small numbers of animals surviving in individual groups in the present experiment, no obvious differences were noted in the frequency or severity of lesions among the various

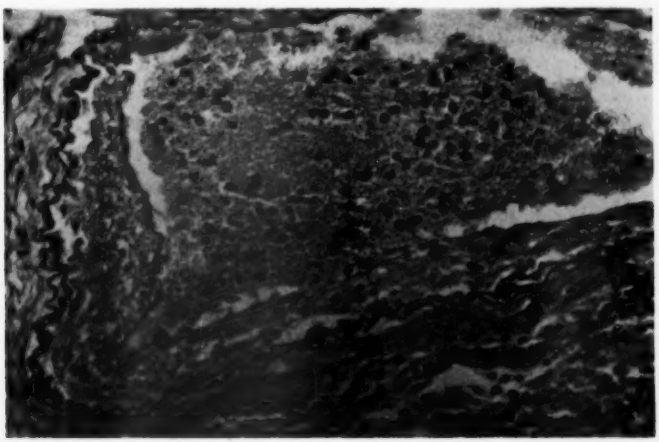


Fig. 10.—A thromboembolic-induced arteriosclerotic lesion of another rabbit. Elastic tissue appears to be forming over the thickest portion of the lesion. This is the characteristic appearance of the plaques produced by the organization of thrombi, and this appearance is also common in arteriosclerotic lesions of man. Aldehyde fuchsin-Van Gieson-hematoxylin; reduced to 69% of mag. × 500.

groups. Except for one organizing thrombus, no lesions were demonstrated in any of the animals of Groups VIII and IX that were not given intravenous clot suspension.

Comment

Certainly no documentation is necessary for the fact that *occlusive* lesions associated with atherosclerosis in man consist principally of fibrin, fat, and fibrous tissue; the pathologic study of a single patient with even moderately advanced lesions suffices for the demonstration of these components. The problems that now face us involve the mechanism and order of deposition of the components. If deposition of lipids is the initiating event, fibrous tissue proliferation might result from intimal reaction of the fat, and any fibrin present within the lesion might be the result of hemorrhage into the plaque. Occlusive thrombosis could occur as a result of physical changes in the endothelial surface and local hemodynamics. If deposition of fibrin is the initiating event, the fibrous tissue proliferation could result from organization of the thrombotic material, and fat could enter later as a result of an obscure "degenerative process"; occlusive thrombosis could occur simply as a result of progression of the basic process. No one has previously suggested that the deposition of fat and of fibrin might occur simultaneously, but this possibility seems feasible in the light of the results of the present experiments.

The significant observation of this study appears to be the rapid appearance, in only a few days, of stainable lipid in pulmonary arterial thromboemboli in rabbits on high-fat diets, while water-fed animals failed to demonstrate the presence of fat in similarly produced lesions. An arterial lesion beginning as a fibrin thrombus can absorb serum lipids in quantities readily demonstrable microscopically. Hence new evidence has been obtained indicating that the demonstration of fat in an "early" arteriosclerotic plaque in man does not necessarily mean that deposition of fat was the initiating event. The thromboemboli injected into the rabbits did not contain demonstrable lipid in appreciable amounts, except in rabbits fed dietary supplements of fat. This fat was present in lesions in all stages of organization, indicating that lipid can be present in thrombi throughout their progressive organization to fibrous intimal plaques.

That the thromboembolic-induced pulmonary arterial lesions obtained in rabbits on a Chow diet only rarely contained fat and were, therefore, unlike human lesions in this respect is not surprising. The usual laboratory ration for animals is low in fat (about 2% by weight), and the usual human diet is comparatively high (about 40% by calories). In approaching the experimental reproduction of pathologic lesions of man that are likely to be related to deposition of fats or other constituents of the blood (for example, atherosclerosis and diabetic glomerulosclerosis), it would seem necessary that the animals be maintained on a diet high in fat which is comparable to the diet of man.

Summary

Small amounts of stainable fat are occasionally present in thromboembolic-induced pulmonary arterial lesions of rabbits on the usual low-fat laboratory diet of Purina Chow. The Chow diet is quite unlike the usual high-fat diet of man and is probably not suitable for the reproduction of pathologic lesions of man characterized by the deposition of lipids. A high intake of dietary fat, near the limits of tolerance, produces a marked but not uniform accumulation of lipid in thrombi. The fat is seen within the first few days after the lodgment of the thromboemboli and is present through all stages of organization of the lesions to the formation of fibrous intimal plaques.

These observations suggest that atherosclerotic lesions of man could also result from the deposition of lipids and thrombi in close association, rather than singly.

Department of Pathology, Washington University School of Medicine, Euclid Ave. and Kingshighway (10).

REFERENCES

1. Katz, L. N., and Stamler, J.: Experimental Atherosclerosis, Springfield, Ill., Charles C. Thomas, Publisher, 1953.

2. Duguid, J. B.: The Etiology of Atherosclerosis, Practitioner 175:241-247, 1955.

3. Hartroft, W. S., and Thomas, W. A.: Pathological Lesions Related to Disturbances of Fat and Cholesterol Metabolism in Man, J.A.M.A. 164: 1899-1905, 1957.

4. Thomas, W. A., and Hartroft, W. S.: Myocardial Infarction in Rats Fed Diets Containing High Fat, Cholesterol, Thiouracil and Sodium Cholate, Circulation 19:65-72, 1959.

5. Keys, A.; Buzzini, R.; Grande, F., and Anderson, J. T.: Effects of Meals of Different Fats on Blood Coagulation, Circulation 15:274-279, 1957.

6. Thomas, W. A.; O'Neal, R. M., and Lee, K. T.: Thromboembolism, Pulmonary Arteriosclerosis and Fatty Meals, A.M.A. Arch. Path. 61:380-389, 1956.

7. Thomas, W. A.; Konikov, N.; O'Neal, R. M., and Lee, K. T.: Saturated Versus Unsaturated Fats in Experimental Arteriosclerosis: Comparison of the Effect of Various Types of Fatty Meals on Thromboembolic-Induced Pulmonary Arteriosclerosis in Rabbits, A.M.A. Arch. Path. 63:571-575, 1957.

8. Wartman, W. B.; Jennings, R. B., and Hudson, B.: Experimental Arterial Disease: I. Reaction of the Pulmonary Artery to Minute Emboli of Blood Clot, Circulation 4:747-755, 1951.

9. Jones, R.; Thomas, W. A., and O'Neal, R. M.: Carbowax Embedding of Tissues, Am. J. Clin. Path. 31:453-466, 1959.

Glomerular Abnormalities in Tuberculosis

LEONARD B. BERMAN, M.D.; TATIANA T. ANTONOVYCH, M.D., and JAMES DUKE, M.D.,
Washington, D.C.

The usefulness of percutaneous biopsy of the kidney for both research and clinical purposes has been abundantly demonstrated in the past eight years.[1] The procedure is uniquely applicable to the study of renal diseases characterized by minimal or absence of clinical manifestations, reversible lesions, and an infrequent or long-delayed fatal outcome. We therefore applied it to an investigation of patients with pulmonary tuberculosis, hoping to study the morphology of early renal tuberculosis. It soon became apparent that the study might more profitably be directed toward the occurrence and pathogenesis of acute alterations in the glomeruli of tuberculous humans.

Historical Background

The term "tuberculous nephritis" is a convenient starting point, for its long and uneven career accurately reflects the confusion and controversy that have surrounded the renal manifestations of tuberculosis. It was sometimes used, on the one hand, to denote a glomerulonephritis specifically related to Mycobacterium tuberculosis or its products.[2] More frequently, it was used to describe discrete foci of parenchymal inflammation supposedly due to the action of the tubercle bacillus.[3] With indiscriminate application to any tuberculous patient showing proteinuria, it eventually came to include such diverse pathologic states in the kidney as amyloidosis, tuberculosis, and pyelonephritis, and, in truth, any other coincidental cause of proteinuria. The reality of a true tuberculous glomerulonephritis remained questionable and poorly defined in spite of some dogmatic statements to the contrary.[4]

One of the early attempts at clarification was made by Holten,[2] in 1924. He and his contemporaries made clinical diagnoses of glomerulonephritis in from 0.5% to 4.5% of patients with pulmonary tuberculosis. These diagnoses were often without convincing clinical features and almost always lacked tissue confirmation. In spite of what really amounted to evidence against a relationship between the two diseases, this author would not exclude the possibility that the tubercle bacillus was a cause of a diffuse glomerulonephritis.

A somewhat more satisfactory study was reported by Lieberthal and Hüth[3] in 1933. Kidneys removed surgically from 1,000 patients with renal tuberculosis were examined microscopically. The authors concluded that the tubercle bacillus was not etiologically related to glomerulonephritis and that the "occasional" case of glomerulonephritis seen was due to secondary invasion by a Streptococcus. Other studies in humans[5] and experimental animals[6] supported this view.

Modern authorities either do not comment on glomerulonephritis in tuberculosis

Submitted for publication Jan. 31, 1959.

Presented in part before the Southern Society for Clinical Research, New Orleans, Jan. 23, 1959.

From the Department of Medicine, Kidney Research Laboratory, Georgetown Division, District of Columbia General Hospital, and the Department of Pathology, Georgetown University Medical Center.

Assistant Professor, Department of Pathology, Georgetown University Medical Center (Dr. Antonovych). Resident, Pulmonary Disease Division, District of Columbia General Hospital; Fellow, American Trudeau Society (Dr. Duke).

Director, Kidney Research Laboratory, Georgetown Division, District of Columbia General Hospital; Assistant Professor of Medicine, Georgetown University Medical Center (Dr. Berman). Work done during tenure of a postdoctoral fellowship, National Heart Institute, National Institutes of Health, Bethesda, Md. Present address of Dr. Berman: Mount Alto Veteran Administration Hospital, Washington, D.C.

or simply mention their occasional coexistence.[7] The weight of available evidence, therefore, heavily favors the absence of a relationship between the two diseases. This is true in spite of occasional case reports, such as that of Abezhous.[8] This author described two children with tuberculosis and a nephrotic syndrome. Her assertion that an underlying glomerulonephritis was responsible for the nephrotic syndrome and that the glomerulonephritis was itself a complication of tuberculosis would have been considerably strengthened by histologic studies.

The purpose of the present paper is to present and discuss the results of clinical evaluation, laboratory examination, and percutaneous biopsy of the kidney in 12 patients with proved tuberculosis, pulmonary and otherwise.

Materials and Methods

The subjects were hospitalized patients chosen at random from the Pulmonary Disease Division, District of Columbia General Hospital. They ranged in age from 20 to 67 years; seven were male and five female; all were Negroes. All 12 patients had sputum cultures positive for M. tuberculosis; in addition, one had the organisms present in a section of ileum removed surgically. Urine cultures were positive for M. tuberculosis in three patients and negative in nine. All had positive tuberculin skin tests. These and other clinical details are recorded in the accompanying Table. The classification of the pulmonary lesions follows the recommendations of the National Tuberculosis Association.

At the time of biopsy, 11 patients had been on antituberculous treatment for weeks or months. The drugs included streptomycin, aminosalicylic acid, isoniazid in various forms, thiocarbanidin [84-isobutory-4-(2-pyridyl) thiocarbanilide] and Hinconstarch * (starch-hydrazide-thiosemicarbasone). Isoniazed and its derivatives were the only compounds common to the 11 patients. The 12th patient was begun on therapy immediately after the biopsy.

Renal biopsy was preceded in all cases by a plain film of the abdomen or an excretory urogram. Bleeding, clotting, and prothrombin times were normal in all. A modified Vim-Silverman needle was used, with the patient in the prone position. Local procaine was the only anesthetic used. There were no complications. The biopsy specimen was immediately fixed in buffered formalin. Sections 3μ in thickness were cut and stained with hematoxylin and eosin, the periodic acid-Schiff reagent, and with the Ziehl-Neelsen technique. The average number of glomeruli per biopsy specimen was 28, with a range of 6 to 61.

Results

A. Histologic Findings.—The biopsy samples from Cases 2 and 10 were normal. The biopsy specimen from Case 12 showed extensive and severe destruction of renal

* Supplied by Dr. George Mast, Nepera Chemical Company, Yonkers, N.Y.

Clinical Data on Twelve Patients with Tuberculosis

Case No.	Age, Yr.	Sex	Extent of TB	Sputum Culture	Urine Culture	P. P. D.	Blood Pressure	Comment
1	43	M	Mod. adv. pulm.	+	−	+	120/80	
2	53	M	Far-adv. pulm.; epididymis, seminal vesicles	+	−	+	120/80	Addisonian on replacement therapy
3	55	M	Miliary; liver (biopsy); epididymis	+	−	+	240/120	Addisonian on replacement therapy
4	67	M	Med. adv. pulm.	+	−	+	105/60	Ca of pancreas
5	32	F	Far-adv. pulm.; kidney	+	+	+	90/50	Portal cirrhosis
6	38	F	Minimal pulm.	+	−	+	155/100	Diabetes mellitus
7	31	F	Mod. adv. pulm.; ileum	+	−	+	130/60	--
8	20	M	Miliary; meninges, epididymis	+	−	+	118/68	--
9	38	M	Miliary; meninges, liver	+	−	+	110/75	--
10	27	F	Far-adv. pulm.	+	+	+	110/70	--
11	50	M	Far-adv. pulm.	+	−	+	130/80	Rheumatic heart disease; portal cirrhosis; pyelonephritis
12	26	F	Minimal pulm.; kidney	+	+	+	150/105	--

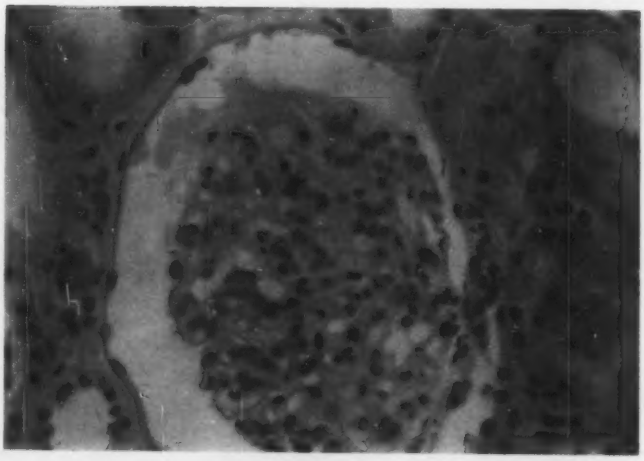


Fig. 1. — Renal biopsy sample. A normal glomerulus showing a uniformly thin basement membrane and a relative paucity of cellular elements. Red blood cells are seen within the capillaries. Protein-containing fluid is seen in the capsular space. Reduced to 62% of mag. × 300.

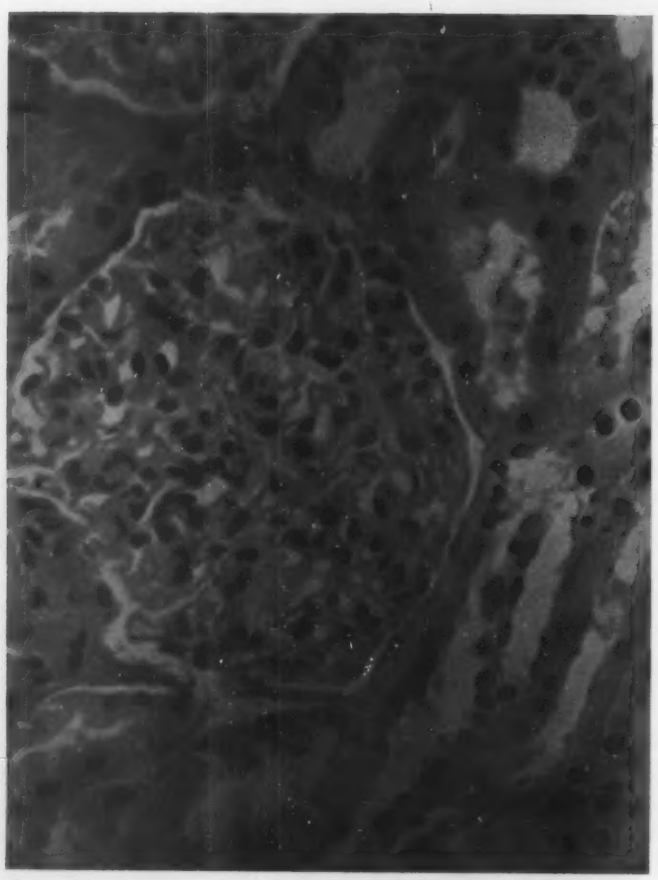


Fig. 2. — Renal biopsy tissue. The glomerulus is enlarged and slightly hypercellular, predominantly with mononuclear elements and some polymorphonuclear leukocytes. There are diffuse thickening of the basement membrane and glomerulocapsular synechiae. Reduced to 85% of mag. × 300.

tissue, with caseating granulomata, and will not be further considered.

The remaining nine biopsy tissues were characterized by thickening and hypercellularity of the glomerular basement membrane of varying degrees. The affected glomeruli comprised 50%-100% of the glomeruli from any one biopsy specimen. The basement-membrane thickening occurred in patchy and diffuse forms, often leaving the capillary loops patent, but devoid of red blood cells. Hypercellularity, composed chiefly of mononuclear elements, exhibited the same variability. Polymorphonuclear leukocytes were infrequently noted in the tufts, as were glomerulocapsular synechiae. Crescent formation was not seen. Arteriolar nephrosclerosis, although occasionally present, was never of such a degree as to produce glomerular changes.

Discrete cortical tubercles were present in Case 9, with surrounding areas of parenchyma which were normal except for the glomeruli. Acid-fast organisms were present in these tubercles. Figures 2-5, from Cases 6, 7, 9 and 8, respectively, illustrate the types of lesions described. Figure 1 is that of a normal glomerulus, for comparison. None of the sections were stained for amyloid. The changes seen did not suggest this entity in any of the biopsy samples.

B. Clinical Correlation.—A clinical diagnosis of glomerulonephritis was made in only one instance (Case 7) on the basis of 18 hours of anuria, followed by the excretion of protein, red cells, and red-cell casts

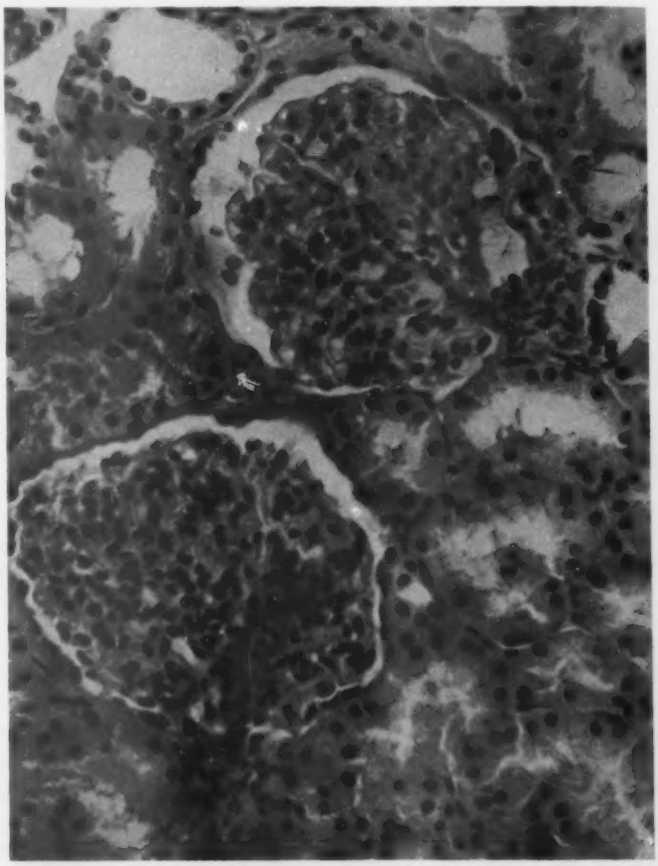


Fig. 3.—Renal biopsy tissue. The two glomeruli are ischemic and hypercellular, with mononuclear and polymorphonuclear cells. Red blood cells lie free in Bowman's space. Reduced to 85% of mag. × 200.

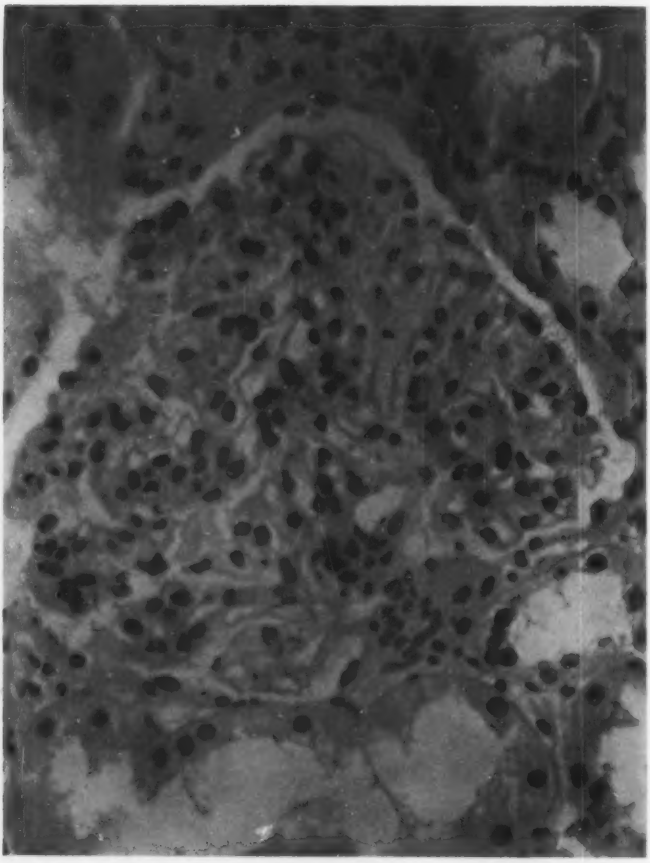


Fig. 4. — Renal biopsy tissue. The glomerulus is enlarged and hypercellular. The basement membrane is focally thickened. The juxtaglomerular apparatus shows scattered polymorphonuclear leukocytes. Reduced to 85% of mag. × 300.

for a 24-hour period. Hypertension and edema did not occur. The blood urea nitrogen reached 90 mg/100 ml. and then returned to normal. This patient had pulmonary and ileal tuberculosis and was admitted with intestinal obstruction. The abnormalities of urine volume and sediment occurred prior to any antituberculous therapy. The biopsy was performed several weeks later, during treatment with streptomycin and thiocarbanidin. Clinical signs of renal disease in the remaining patients were limited to hypertension and/or slight proteinuria in 5 of the 11 cases.

Of the two patients showing cortical tubercles, one (Case 8) had miliary tuberculosis with normal excretory urograms and negative urine cultures for M. tuberculosis. The other (Case 12) had extensive pulmonary tuberculosis, calyceal destruction, seen by pyelography, and a urine culture positive for M. tuberculosis. As may be seen from the Table, the extent of the tuberculosis varied from minimal to far advanced in the entire group. This parameter bore no obvious relationship to the glomerular abnormalities found.

Comment

The glomerular changes illustrated in Figures 2-5 resemble, in varying degrees, those seen in poststreptococcal glomerulonephritis [7,9] and the glomerulonephritis of lupus.[10,11] Whether the changes in the tuberculous patients should be labeled glomerulonephritis is a problem not easily settled.

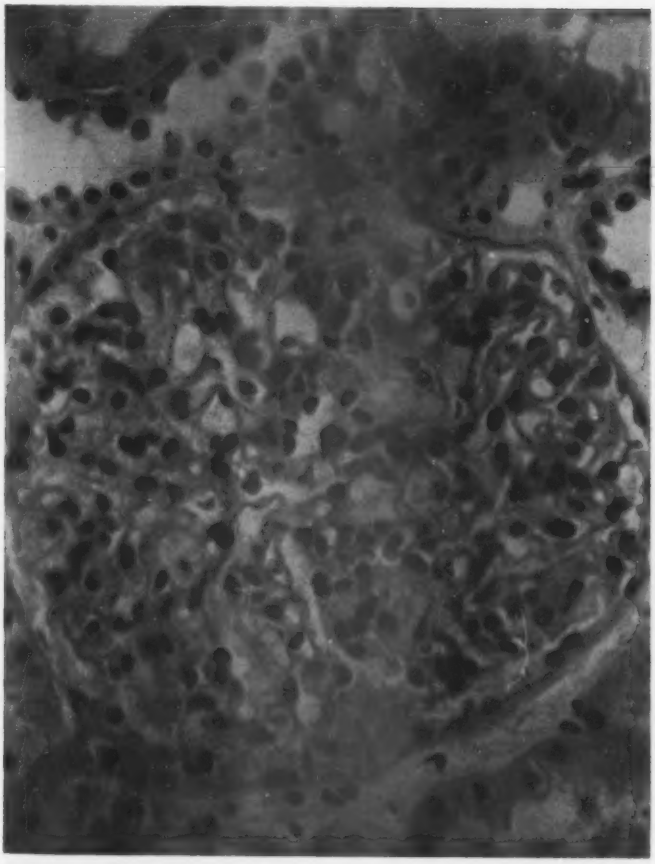


Fig. 5. — Renal biopsy tissue. The glomerulus obliterates Bowman's space. It is ischemic and hypercellular. The basement membrane is focally thickened. Reduced to 85% of mag. × 300.

If the term may be used to signify a histologic alteration without an implication of a corresponding clinical state, it might then be reasonable to speak of tuberculous glomerulonephritis. This problem will become increasingly acute as biopsy data accrue.[12]

Several possible explanations of the findings in our patients can be considered. Coincidence of a poststreptococcal glomerulonephritis with tuberculosis in 9 of 12 patients seems unlikely. The possibility of antituberculous drugs as etiologic agents may be dismissed in view of the fact that Patient 4 had not received any at the time of biopsy and Patient 7 showed clinical signs of glomerulitis before treatment was begun. Toxicity studies on isoniazid, the only drug common to all patients in this series, include no well-documented nephrotoxic effects, or tissue demonstration of glomerular changes in man.[13] Rats may show slight granular degeneration of the proximal convoluted tubules on long-term therapy.[14] Glomerular lesions have been described by Barr [15] and by others, in association with hepatic cirrhosis. The description of such lesions suggests no similarity with those found in our patients. Furthermore, only Patients 5 and 11 were known to be cirrhotic. Liver disease thus seems an unlikely explanation.

Earlier writers, interested in the hypersensitivity associated with tuberculosis, had speculated on the possibility of allergic re-

actions within the kidneys, as well as other organs of tuberculous patients, perhaps in response to liberation of products from tuberculous tissue elsewhere in the body.[16,17] Several lines of evidence may be cited in support of this hypothesis. First, acute glomerulonephritis, clinically and histologically similar to human disease, can regularly be produced in animals by immunologic means, such as anti-kidney-serum or foreign-protein injections.[18] Of even more significance in the present discussion is the production of acute glomerulonephritis by the injection of tuberculin into the renal artery of tuberculous swine.[16] Control animals failed to react in a similar fashion. Evidence is also available to suggest an "allergic irritability" or heightened antibody formation in animals infected with tuberculosis and given nontuberculous antigens.[19,20] Finally, secondary amyloidosis, well known as a complication of tuberculosis, exhibits many of the same characteristics of an antigen-antibody reaction as does glomerulonephritis when studied by antibody-labeling techniques.[21]

We therefore suggest that the lesions found in our patients are manifestations of glomerular hypersensitivity to tuberculous products occurring in sensitized (tuberculous) subjects.

The discrepancy between the incidence of glomerulonephritis in tuberculosis reported in earlier series and the present findings is striking. This may be partly due to the small number of patients, but may also be related to the advantages of biopsy over autopsy material. Early sampling is chief among these, for the glomerular changes in the present group are probably acute and reversible. It is possible that many of these changes will have disappeared at the time of autopsy. Biopsy tissue also offers considerably finer delineation of glomerular structure and, consequently, increases the chances of diagnosing milder lesions.[10] Finally, the recognition of glomerular abnormalities may be rather difficult, even with biopsy material. The thickness of the sections and the experience of the histologist are important variables. Work recently completed has provided us with a useful experience in comparing normal kidney biopsy tissues with those of the tuberculous patients.[22]

The results reported in this paper offer strong encouragement to the aggressive search for glomerular inflammation in a large number of diseases not presently considered as etiologic factor of glomerulonephritis. The rewards include the solution of difficult diagnostic problems and the promise of adding to the understanding of an important group of disease states.

Summary

Twelve patients with tuberculosis were randomly chosen for study by kidney biopsy. Nine were found to have varying degrees of thickening and hypercellularity of the glomerular basement membrane. The affected glomeruli comprised from 50% to 100% of the glomeruli in each biopsy specimen. The pathogenesis of these lesions may be related to glomerular hypersensitivity to tuberculous products.

We gratefully acknowledge the cooperation and support of Dr. Sol Katz, Chief Medical Officer, Pulmonary Disease Division, District of Columbia General Hospital, and Dr. Charles F. Geschickter, Professor of Pathology, Georgetown University Medical Center.

Department of Medicine, Georgetown University Hospital.

REFERENCES

1. Brun, C., and Raaschou, F.: Kidney Biopsies, Am. J. Med. 24:676, 1958.

2. Holten, C.: Nephritis Caused by Tuberculosis, Acta med. scandinav. 61:107, 1924.

3. Lieberthal, F., and Hüth, T.: Tuberculous Nephritis and Tuberculous Bacilluria, Study of 1,000 Operated Cases of Renal Tuberculosis; Pathology; Bacteriology, J. Urol. 30:153, 1933.

4. Thomas, G. J.: Renal Tuberculosis, J.A.M.A. 94:229, 1930.

5. Medlar, E. M.: Renal Infection in Pulmonary Tuberculosis: Evidence of Healed Tuberculous Lesions, Am. J. Path. 2:401, 1926.

6. Medlar, E. M., and Sasano, K. T.: Experimental Renal Tuberculosis, with Special Reference

to Excretory Bacilluria, Am. Rev. Tuberc. 10:370, 1925.

7. Fishberg, A. M.: Hypertension and Nephritis, Ed. 5, Philadelphia, Lea & Febiger, 1954.

8. Abezhous, A. M.: Treatment of Diffuse Glomerulonephritis of Tuberculous Origin in Children, Pediatrija (Russian), No. 3, May-June, 1955, p. 38.

9. Brun, C.; Gormsen, H.; Hilden, T.; Iversen, P., and Raaschou, F.: Kidney Biopsy in Acute Glomerulonephritis, Acta med. scandinav. 160:155, 1958.

10. Muehrcke, R. C.; Kark, R. M.; Pirani, C. L., and Pollak, V. E.: Lupus Nephritis, Baltimore, Williams & Wilkins Company, 1957.

11. Berman, L. B., and Schreiner, G. E.: Clinical and Histologic Spectrum of the Nephrotic Syndrome, Am. J. Med. 24:249, 1958.

12. Berman, L. B.: A Classification of Glomerular Diseases, Bull. Georgetown Univ. M. Center 12:200, 1959.

13. Elmendorff, D. F., Jr.; Cawthon, W. U.; Muschenheim, C., and McDermott, W.: The Absorption, Distribution, Excretion, and Short Term Toxicity of Isonicotinic Hydrazide (Nydrazid) in Man, Am. Rev. Tuberc. 65:429, 1952.

14. Rubin, B.; Hassert, G. L., Jr.; Thomas, B. G. H., and Burke, J. C.: Pharmacology of Isonicotinic Acid Hydrazide (Nydrazid), Am. Rev. Tuberc. 65:392, 1952.

15. Barr, R. W., and Sommers, S. S.: Endocrine Abnormalities Accompanying Hepatic Cirrhosis and Hepatoma, J. Clin. Endocrinol. 17:1017, 1957.

16. Long, E. R., and Finner, L. L.: Experimental Glomerulonephritis Produced by Intrarenal Tuberculin Reactions, Am. J. Path. 4:571, 1928.

17. Seligson, F.: Poncet's Disease: Clinical Observations on Inflammatory and Degenerative Joint Reactions to Tuberculosis, Am. Rev. Tuberc. 52: 463, 1945.

18. Seegal, B. C., and Bevans, M.: The Production of Glomerulonephritis by Immunologic Methods, J. Chron. Dis. 5:153, 1957.

19. Lewis, P. A., and Loomis, D.: Allergic Irritability: Formation of Anti-Sheep Hemolytic Amboceptor in Normal and Tuberculous Guinea Pigs, J. Exper. Med. 40:503, 1924.

20. Dienes, L.: Further Observations Concerning Sensitization of Tuberculous Guinea Pigs, J. Immunol. 15:153, 1928.

21. Mellors, R. C., and Ortega, C. G.: Analytical Pathology: III. New Observations on the Pathogenesis of Glomerulonephritis, Lipid Nephrosis, Periarteritis Nodosa, and Secondary Amyloidosis in Man, Am. J. Path. 32:455, 1956.

22. Berman, L. B.; Antonovych, T., and Tublin, I.: The Normal Kidney Biopsy in Humans, in preparation.

Stimulation of Granulation Tissue Growth by Tissue Extracts

Study in Intramuscular Wounds in Rabbits

ROBERT H. EDWARDS, M.D.; SEVERINO S. SARMENTA, M.D., and GEORGE M. HASS, M.D., Chicago

Introduction

Factors responsible for proliferation of cells in the healing of wounds are not known. Growth-promoting factors for cells have been demonstrated in many tissue homogenates and extracts.[1-3] In the study of these factors, investigators have used cells in tissue culture and have determined the effect of various synthetic and natural media on their maintenance, growth, and migration. To date, the sustained growth stimulus of embryo extracts with plasma has not been surpassed by other media, although certain adapted cell lines may grow very well in chemically defined media, to which no natural tissue extracts have been added.[4,5] There is considerable similarity in the behavior of fibroblasts proliferating in vivo and in vitro.[6] The studies in vivo have been less successful than those in vitro in analyzing factors affecting wound healing.

Study of acceleration of wound healing by local application of various substances usually has involved planimetric measurement of repair of cutaneous wounds.[7] This method has been criticized as unreliable and is fraught with difficulties, one of which is contraction.[8] Contraction, an inherent part of healing, adds a greatly variable element to the interpretation of rate of restoration of continuity between separated edges of wounds. In some wounds the reduction in spatial discontinuity is largely due to contraction.[8-10] Planimetry also encounters another source of error, due to variable epithelization, which is difficult to measure.[8] Reproducing experiments with this technique is also difficult, since the size of the wound affects the rate of healing.[11-15] The validity of this conclusion, however, has been questioned.[8,59] The planimetric technique can be further criticized because of frequent infection, due to superficial location of the wound.

Measurement of tensile strength either by determination of pressure necessary to disrupt a viscus by fluid under pressure[16] or by measuring the force needed to pull a wound apart[17-19] aids in determination of effects of various substances or conditions on wound healing. Here, the basic factors in healing of most wounds, namely, the vascularized proliferation of fibroblasts and associated production of collagen fibers, are in effect all that are being measured.[20,21] Although epithelial-cell migration also occurs, it has little to do with the strength of the healing wound, for the tensile strength of united surfaces is dependent principally on collagenous connective tissue repair.[20,22] Measurement of tensile strength has several limitations. It is not easily reproduced in control animals, and the pressure to disrupt a wound may vary directly with the diameter of the viscus and is not wholly dependent upon the manometer reading.[23]

A unique method for quantitative measurement of healing of a wound is the determination of changes in electrical potential across a wound as healing progresses.[24]

Submitted for publication July 20, 1959.

From the Department of Pathology, Presbyterian-St. Luke's Hospital.

We gratefully acknowledge support by the National Institutes of Health (2G-129, H-1630), the Leukemia Research Foundation, Inc., and the Otho S. A. Sprague Memorial Institute.

This method has received the same criticisms as those depending on surface measurements, and for the same reasons.

Another method involves intracardiac injection of carbon particles in colloidal suspension, followed by observing vascularization of experimental and control wounds in the same animal.[25]

We used a method for measurement of connective tissue growth and development under conditions which did not involve epithelization, wound contraction, or infection, and which could be suitably controlled. In 1951 Grindlay and Waugh[26] inserted a polyvinyl alcohol sponge (Ivalon) into subcutaneous tissues of animals. This sponge acted as a framework for granulation tissue which permeated its pores. There was interest in the use of the sponge as a prosthesis[26,29] in surgical repair, but it was shown[30-34] that the sponge not only elicited a foreign-body reaction but also was slowly absorbed by phagocytic action. Fortunately, during the first two weeks of granulation-tissue ingrowth and differentiation into mature connective tissue, the foreign-body reaction and absorption are minimal, and we have found the sponge useful for our purposes.

Materials and Methods

Young adult male and female rabbits, each weighing about 6 lb., were used for assay of the fibroblastic-collagenous reparative response. Ivalon sponges were prepared by the methods of Grindlay and Waugh,[26] cut into cylindrical form, measuring 1.5 cm. in diameter by 2.5 cm. in length (Fig. 1), and autoclaved. The tissue extracts were prepared from eight-day chick embryos, autologous rabbit spleen, or autologous granulation tissue removed from thigh muscles five days after crushing. These natural tissue extracts support the growth of fibroblasts in vitro.[35-44] The extracts were prepared by homogenization of the tissue under sterile conditions in Tyrode's solution at a concentration of tissue wet weight: aqueous medium (1:10). Heparinized plasma was prepared from the animal to be used for assay of the extract. This was mixed in equal parts with the tissue extract to be assayed. The dry experimental sponge was immersed in this mixture. All air bubbles were extruded from the immersed sponge by mechanical pressure, and within a minute or two the mixture formed a solid clot throughout the pores of the sponge. At the same time a control sponge containing a mixture of equal quantities of plasma and Tyrode's solution was prepared, and time was allowed for a clot to form throughout the interstices of the sponge.

Fig. 1.—Ivalon sponge cut into cylindrical form prior to implantation into dorsal muscles of rabbit.

Twenty rabbits were used for each of the three extracts. The freshly clotted sponges were implanted under local anesthesia in the paravertebral muscles through a single midline skin incision. Each sponge was embedded between longitudinal fibers of muscle which had been carefully separated, 3 cm. lateral to the skin incision. The control and experimental sponges were inserted at the same level, since in rabbits healing occurs more rapidly as the head is approached.[45] Closure was made in multiple layers by absorbable surgical (gut) sutures. The sponges were left in situ for periods varying from two days to three weeks, at which time the animals were killed. The sponges and surrounding tissues were fixed in formalin. Blocks through the midtransverse axis of the sponges were embedded in paraffin. Cross sections for microscopic study were stained with hematoxylin and eosin, as well as with Mallory's aniline

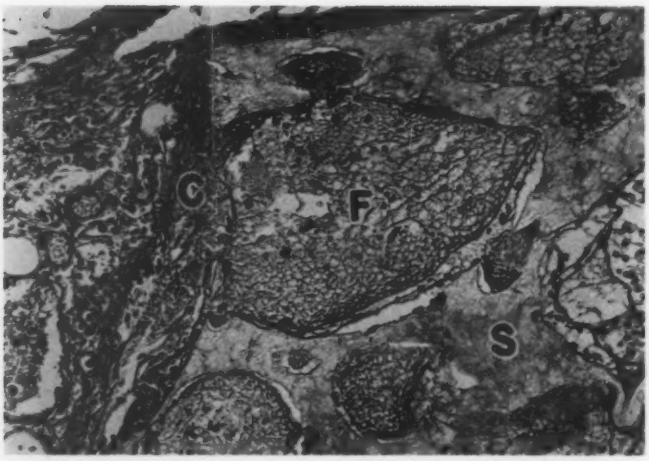


Fig. 2.—Peripheral area of sponge containing tissue extract three days after implantation. Many fibrin strands, supporting a few inflammatory cells and occasional fibroblasts, are present in the sponge pores. *C*, early capsule formation at margin of sponge; *S*, Ivalon sponge trabecula; *F*, sponge pore containing fibrin and cells. Hematoxylin-eosin stain; reduced to 60% of mag. × 320.

blue connective tissue stain.[46,47] The effects of the Tyrode solution-tissue extract-plasma medium and its control Tyrode solution-plasma medium were compared by microscopic determination of (*a*) the extent of penetration of granulation tissue[21,48] from the margin toward the center of the sponge and (*b*) the amount and maturity of collagen fibers.[49,50]

Infection was not a problem.[51,52] All animals were healthy and gained weight during the period of observation.

Results

Immediately following implantation there was minor hemorrhage around the sponges. This did not permeate the peripheral pores, for these were already filled with clotted plasma. Two days later, there was marked fibroblastic proliferation directly adjacent to the sponges, and occasional fibroblasts were found invading the clot in the marginal peripheral interstices. The presence of fibroblasts at this time coincides with their earliest appearance in granuloma formation.[6,50] In this region there was also a thick band of eosinophilic material composed of condensed fibrin.[32] The more delicate fibrin strands were arranged in a loose meshwork within the interstices of the sponge (Fig. 2). Fibroblasts which had migrated here had a tendency to elongate and grow along these fibrils, which served as support for their migration.[6,53] In sponges containing no tissue extract, the pe-

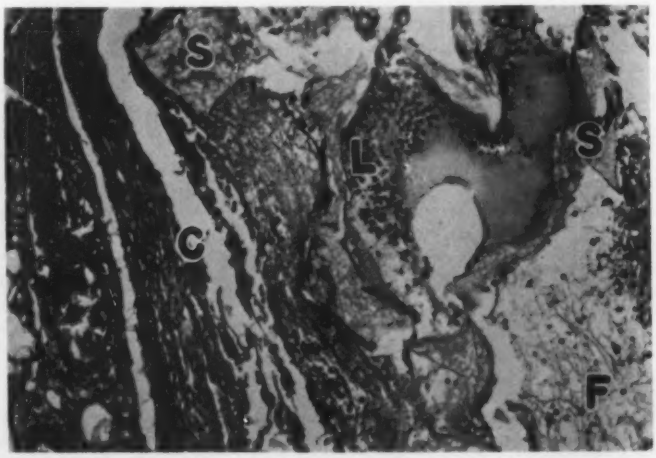


Fig. 3.—Peripheral area of control sponge three days after implantation. Fibrin and leukocytes are numerous in sponge pores, and fibrous encapsulation of sponge is conspicuous. There are no fibroblasts in the sponge pores. *S*, Ivalon sponge; *F*, fibrin in pores; *L*, leukocytes in pores; *C*, capsule. Hematoxylin-eosin stain; reduced to 60% of mag. × 150.

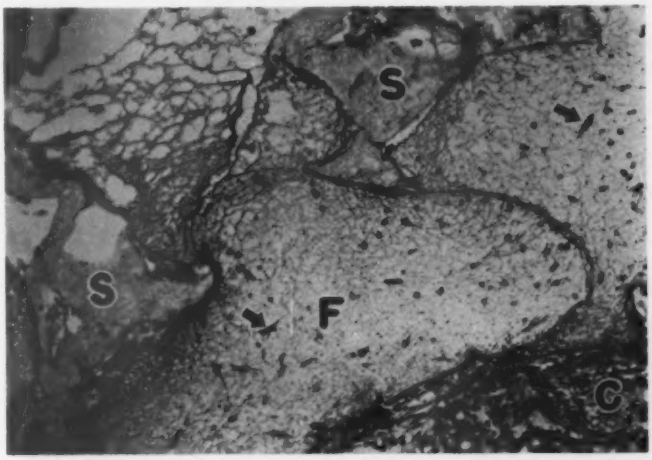


Fig. 4.—Peripheral portion of sponge saturated with tissue extract and implanted for three days. Fibroblasts are numerous. *F*, fibrin; *S*, Ivalon sponge; *C*, capsule; arrows indicate fibroblasts. Hematoxylin-eosin stain; reduced to 60% of mag. × 150.

ripheral sponge pores contained residual leukocytes, wandering macrophages, and fibrin (Fig. 3). In sponges saturated with tissue extract the concentration of the fibroblasts in the peripheral pores was consistently increased (Fig. 4). This was noted after two to three days in vivo in all sponges containing any one of the three tissue extracts. At this early stage of growth of fibroblasts into the sponges, the greatest penetration was in peripheral pores adjacent to small nerves caught in the granulation tissue about the sponge. This may be related to the fact that in some tissues of lower animals regeneration does not occur in the absence of a nerve supply.[54-56] This may also be related to the increased vascularity about the nerve bundles, since proximity to blood supply stimulates granulation-tissue proliferation into wounds.[8,57]

By five days there was a conspicuous fibroblastic proliferation, with fibroblasts throughout the peripheral spaces of the sponge. Collagen was demonstrated in these areas at this time, and small proliferating capillaries were plentiful. We never found significant collagen formation in areas devoid of capillary formation, as previously observed.[58,59] Multinucleated cells along the sponge surfaces were apparent by this time. These represented reaction to the sponge as a foreign material.

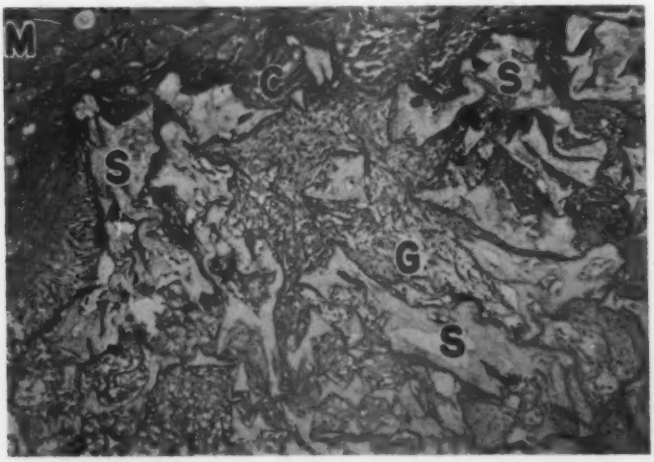


Fig. 5.—Control sponge at seven days, showing extent of penetration of granulation tissue from musculocapsular surface. Compare with Figure 6. *M*, muscle; *C*, capsule; *G*, granulation tissue; *S*, sponge. Hematoxylin-eosin stain; reduced to 60% of mag. × 100.

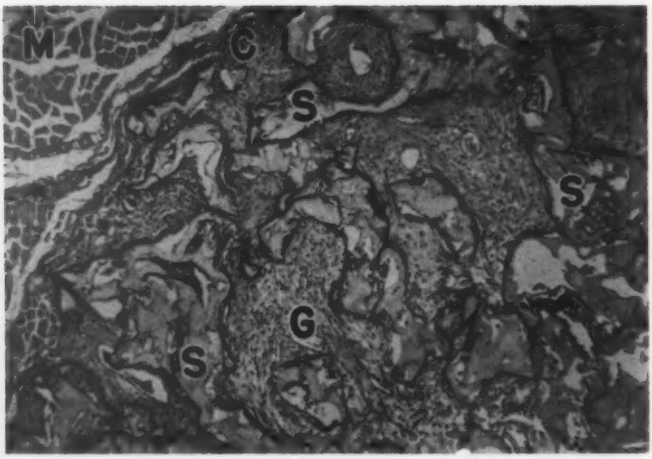


Fig. 6.—Control sponge at 10 days. Note deeper ingrowth of connective tissue and larger capillaries in areas of more mature collagen. Hematoxylin-eosin stain; reduced to 60% of mag. × 100.

By seven days the amount of collagen and the extent of penetration by fibroblasts were more conspicuous (Fig. 5). However, in the peripheral pores adjacent to degenerate skeletal muscle there was no fibroblastic invasion of the sponge. This was consistent with the idea that necrosis of tissue may prolong the lag period of wound healing.[60]

By 10 days there was extensive capillary proliferation and an increased coarseness of most collagen fibers (Figs. 6 and 7). The penetration by granulation tissue was centripetal (Fig. 10). In the central sponge pores into which the granulation-tissue front was advancing, the sequence of events described in the earlier periods for the peripheral pores was repeated, the exudative phase being succeeded by the reparative (fibroblast and capillary proliferation with subsequent collagen deposition) phase, much as has been described for a closed wound.[18] Figure 8 represents the usual ingrowth of granulation tissue and amount of newly formed collagen in a sponge containing tissue extract. The comparable pattern in the control sponge is shown in Figure 9. In some animals there was complete filling of interstices of experimental sponges by vascularized stroma, but there was never complete filling of contralateral control sponges.

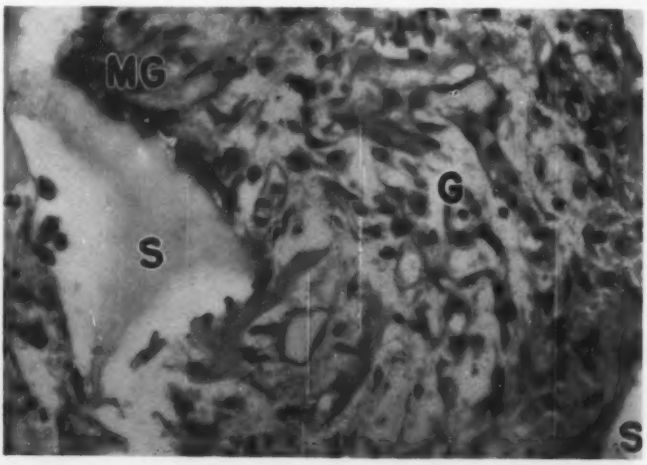


Fig. 7.—Higher magnification of central sponge pore at 10 days. Note marked capillary and fibroblast proliferation with fibrin deposits and multinucleated cells attached to surface of sponge material. *S*, Ivalon sponge; *G*, proliferating capillaries and fibroblasts in pores; *M.G.*, multinucleated giant cells. Hematoxylin-eosin stain; reduced to 60% of mag. × 700.

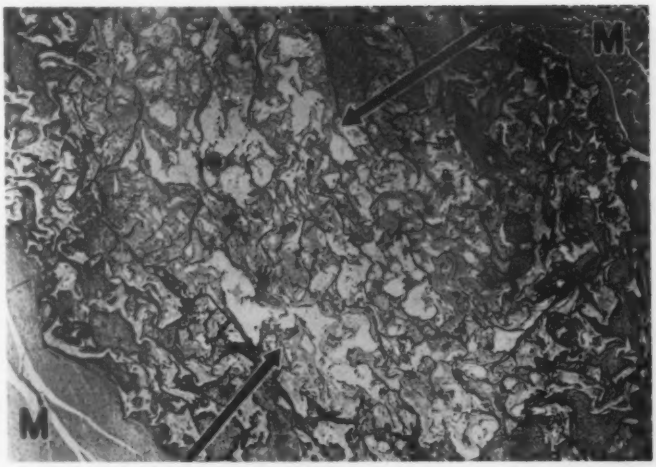


Fig. 8. — Sponge containing tissue extract left in situ for 10 days. Note extent of connective tissue penetration into sponge and compare with Figure 9. Arrows indicate extent of penetration. Hematoxylin-eosin stain; reduced to 60% of mag. × 50.

By 14 days the interstices of sponges were often completely filled by granulation tissue, with mature collagen fibers in all peripheral pores (Fig. 11). In some cases, even at 16 days, the central pores still contained only fibrin clot and inflammatory cells. In these instances of restricted granulation-tissue penetration, each control sponge (Fig. 13) exhibited less penetration than the experimental one containing tissue extract (Fig. 14).

By 21 days, when stromal penetration was always complete in both experimental and control sponges, the experimental sponge still exhibited an increased coarseness of collagen bundles (Fig. 16) as compared with the control sponge (Fig. 15). This was consistent in all cases 10 or more days after implantation. This increased coarseness was the most consistent difference, over and above the extent of stromal penetration, between the sponges with and without tissue extracts. This difference is consistent with the electron microscopic finding of increasing width of collagen fibers in granulation tissue as it matures to adult connective tissue.[50,61]

In sponges saturated with autologous plasma-chick embryo extract, an inflammatory reaction was observed. This consisted

Fig. 9.—Control sponge in same rabbit as was the sponge in Figure 8. Arrows indicate extent of deepest penetration of connective tissue. Hematoxylin-eosin stain; reduced to 60% of mag. × 50.

of clusters of mononuclear cells in pores of sponges where granulation tissue had proliferated four or more days after implantation. This reaction, hardly detectable at four days, increased with time and was maximal about the 16th day. Lymphocytes dominated the reaction, but plasma cells and pigment-laden polymorphonuclear cells and macrophages were also present (Fig. 12). At 21 days some reactions were well circumscribed, with a central core of reticulum cells surrounded by the cells described above.

Having demonstrated accelerated stromal growth in the form of an increased mass of granulation tissue in the presence of chick embryo and adult autologous tissue extracts, we attempted to accelerate the response further by triweekly injections of chick embryo extract percutaneously into a subcutaneously implanted sponge. Microscopic study invariably revealed acute inflammation and necrosis in the sponges, whereas the control sponges, injected triweekly with Tyrode's solution for the same period of time in the same rabbit, always showed the predictable amount of stromal penetration. Bacterial cultures of experimental sponges were consistently negative.

Comment

Attempts to hasten the healing of wounds by the local and parenteral administration of various substances have been made by many investigators. Although some results were encouraging, many conclusions have been criticized either because of the method used or because of inadequate control studies. These criticisms, as well as conflicting results by different people using the same techniques, prompted some investigators to conclude that the healthy organism possesses its own optimal stimulus for tissue growth and repair.[62] There is no doubt that a point exists where no further increase in the rate of wound healing can occur,[63] just as in tissue culture, where proliferation and migration of cells under optimal conditions reach a rate beyond which added stimulatory substances no longer exert an effect. This is probably related to a minimum time necessary for cell reduplication.[2] Yet it does not follow that wound healing cannot be accelerated and otherwise improved by providing substances which the organism is unable to supply at optimal levels at critical periods in the loci where tissue is undergoing repair.[63,64]

An increased rate of wound healing in rats injected intraperitoneally with adult and embryo extracts has been described.[65] Others have recorded equal stimulatory effects by parenteral administration of various tissue extracts.[66] Increased deoxyribonucleic acid (DNA) synthesis in the liver and spleen of rats following injections of ex-

Fig. 10.—Control sponge after 10 days in muscle. All except central sponge pores contain connective tissue. *M*, muscle; *G*, extent of granulation tissue penetration; *C*, capsule. Hematoxylin-eosin stain; reduced to 39% of mag. × 30.

Fig. 11.—Control sponge embedded for 14 days. Note mature connective tissue and many foreign-body giant cells lining sponge material. *M*, muscle; *G.T.*, granulation tissue in pores; *S*, Ivalon sponge; *M.G.*, multinucleated giant cells adjacent to sponge material. Hematoxylin-eosin stain; reduced to 39% of mag. × 100.

Fig. 12.—Sponge containing chick embryo extract embedded for 14 days. Granulomatous reaction consisting for the most part of mature lymphocytes. *M*, muscle; *S*, Ivalon sponge; *G.T.*, granulation tissue; *G.R.*, granulomatous reaction. Hematoxylin-eosin stain; reduced to 39% of mag. × 100.

Fig. 13.—Control sponge after 16 days. Compare connective tissue penetration with that of Figure 14. *M*, muscle; *S*, Ivalon sponge; *C.T.*, connective tissue penetration. Hematoxylin-eosin stain; reduced to 39% of mag. × 40.

Fig. 14.—Sponge containing tissue extract embedded in same rabbit as sponge of Figure 13. Hematoxylin-eosin stain; reduced to 39% of mag. × 40.

Fig. 15.—Control sponge at 21 days stained for collagen. Compare collagen concentration with that of Figure 16. *M*, muscle; *S*, Ivalon sponge; *C.F.*, collagen fibers. Mallory's aniline blue connective tissue stain; reduced to 39% of mag. × 100.

Fig. 16.—Sponge containing tissue extract in same animal as in Figure 15. Note increased coarseness of collagen bundles. Mallory's aniline blue connective tissue stain; reduced to 39% of mag. × 100.

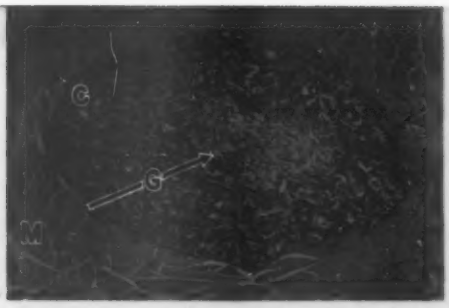


Figure 10

Figure 11

Figure 12

Figure 13

Figure 14

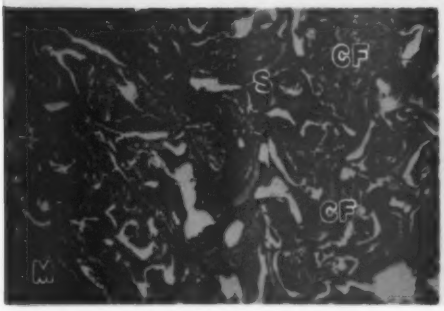


Figure 15

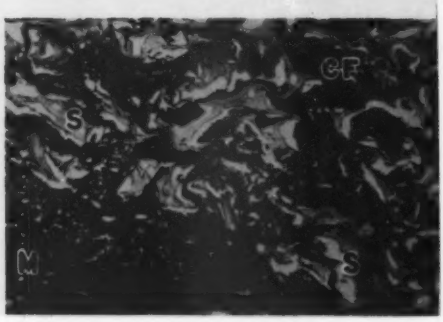


Figure 16

Stimulation of Growth of Granulation Tissue

tracts of rat embryos has been interpreted as due to increased cell growth,[67] but negative results[68] were obtained as gauged by increase in over-all growth and weight in mice following injections of embryo extracts.

Studies of effects of tissue extracts given parenterally are surpassed numerically by clinical and experimental evaluations of effects of local application to wounds of adult and embryo tissue extracts.[69-90] Many of these extracts stimulated growth of cells in vitro. These[72,77,83] appeared to accelerate the healing of wounds in the studies of some investigators, but when applied topically by others,[14,82,91] they failed in many instances to hasten the healing process. It has been suggested[14] that any locally applied substance capable of stimulating healing is probably soluble, thereby interfering with the interpretation of differences between the rate of healing of experimental and control wounds in the same animal. However, it has been our contention[92] that, by trapping added growth-promoting substances in a fibrin clot,[93-95] stimulation of growth will be maximal where these substances are concentrated in the clot, if the concentration is more nearly optimal than that supplied by the animal.

Locally applied substances, other than tissue extracts, may increase the normal rate of healing. Cod liver oil has been found stimulatory,[96] but the increased rate of healing in healthy animals not deficient in vitamin A has been questioned.[63] Several compounds with sulfhydryl groups have been found[97] effective in accelerating the healing process. Thiocresol,[98] methionine,[99] and glutathione and cysteine[100,101] have been described as local stimulants to growth. Chlorophyll applied to wounds apparently decreased the healing time in animals,[102] but no favorable stimulation to healing was reported in man.[103] Artificially induced acidosis hastened wound closure,[104] but this conclusion is questionable.[105]

Certain hormones may influence wound healing and formation of granulation tissue. Desoxycorticosterone acetate (DOCA) and somatotropin stimulate these processes.[106,107] However, most hormones investigated,[108-119] including cortisone, androgenic steroids, thyroxin, and thyrotropin, inhibit healing. The plasma of animals which have undergone partial hepatectomy stimulates mitotic activity in the liver and several other organs.[120-123]

Other studies of wound healing have involved creating nutritional deficiencies in animals to determine the importance of various substances in returning the healing process to normal. Animals on low-protein diets[99,124] or with a low plasma protein level[125,126] show delay in healing, and it has been proved clinically[127,128] that this delay can be corrected by a high-protein diet. Apparently, growth-promoting substances are not mobilized in sufficient concentration in the wound to support normal healing. In this connection, the required substances may not be mobilized optimally even with usual diets, since others[129,130] have demonstrated acceleration of healing by administration of a diet containing more protein than a standard diet. The amount of reparative activity in the wound is only partially dependent upon the diet, because good healing, although retarded, occurs even in fasting animals.[131] Of the known vitamins affecting wound healing, one of the most important is ascorbic acid. Animals with absolute scorbutus[132-136] do not synthesize collagen, although other phenomena associated with wound healing apparently progress satisfactorily.[20]

All wounds heal in essentially the same way, a fixed pattern being followed,[137] whether it be a superficial or a deep wound. The sponge method used by us served as a spatial defect in the animal's tissues and has been likened[31] to a closed wound, except that here there is no need to measure tissue contraction or epithelization. This technique, then, permitted study of only one aspect of the healing process, namely, the growth and differentiation of granulation tissue in a special medium within a standard environment. The advantages of measuring only this one elementary facet of wound healing have been pointed out.[20]

We have, in effect then, a localized, closed wound and also a "test tube" within the animal for the study of the relative effects of substances initially in the "test tube" and those supplied by the host to the "test tube" and its environment. The healing processes observed in the usual type of wound are demonstrable in this method as well. Thus, the lag or latent [69] period described in surface wounds [60] and in viscus-wound-rupture studies [16,99] was demonstrated by this technique, but a more logical end-point to such a period was distinguished, since microscopic observation of fibroblastic activity in less than 48 hours preceded by several days any observable increase in wound tensile strength or diminished wound size. In our experiments with three different tissue extracts, we were able in the first few days following sponge implantation to demonstrate a far greater number of fibroblasts proliferating in the pores of the sponges containing tissue extracts than those without the extracts. Although attempts to shorten the lag period in wound healing failed,[20] this method may prove useful in detecting an earlier appearance of the proliferative (fibroblastic) phase of wound healing.

This method also offers an opportunity to make a controlled study of the later phases of wound healing. Capillary-bud proliferation lagged behind the proliferative migratory invasion by fibroblasts and macrophages, but, as soon as this occurred, new collagen fibers became recognizable in the peripheral interstices of the sponge. It is these reparative processes which lend most support to the healing wound,[21] and it is upon these that transfixed extracts of chick embryo, autologous splenic, and granulation tissues induced acceleration and maturation. The rate of penetration of the proliferating and migratory elements of the ingrowing granulation tissue was consistently increased by the combination of these extracts with the plasma clot in the sponge. Thus, in the early days following the latent period centripetal ingrowth by proliferating capillaries, surrounded by fibroblasts and mononuclear cells, was always greater in the experimental than in the control sponges. Moreover, although we did not detect an earlier deposition of collagen in experimental sponges, sponges containing tissue extracts always exhibited a denser accumulation of coarser fibers of collagen within sponge pores after five days' implantation. This indicated accelerated maturation of collagen fibers and was best demonstrated by comparing the thick, coarse bundles of fibers, approaching scar tissue in characteristics, in the peripheral pores of any sponge embedded for two weeks with the more recently formed fibers nearer the center of the same sponge. This difference was conspicuous up to three weeks following implantation, by which time the interstices of the sponge were occupied by the host's stroma and dense connective tissue had formed throughout both the experimental and the control sponges. However, in the period from two days after implantation until the growing front of granulation tissue had invaded the central sponge pores (usually at 14 days) we were able to detect effects of tissue extracts on growth and differentiation of granulation tissue as described.

Several incidental findings became evident. At any early stage before mature granulation tissue had formed in even the peripheral pores of a sponge, solitary fibroblasts were often found in mitotic division deep in the sponge and far removed from the sponge margin (Fig. 4). Leukocytes and macrophages in varying numbers were always found adjacent to the solitary fibroblasts. Though this finding does not contradict the belief of others [20,31,48,138] that fibroblasts proliferating in a wound originate only from fixed-tissue fibrocytes, we were unable to demonstrate by serial sections any continuity between these dividing cells and the chains of fibroblasts continuous with the host's tissues.

We were also able to demonstrate an atypical inflammatory reaction in the granulation tissue in sponges when chick-embryo extract was used. Collections of mono-

nuclear cells, often in the form of granulomas, were found in the stroma in pores of all sponges containing chick-embryo extract. Identification of all cell types involved in the reaction was difficult, but they were predominantly lymphocytes in the early stages, while later there were increasing numbers of plasma cells. In sections taken at 21 days, central aggregates of reticulum-like cells appeared as germinal centers surrounded by the above-mentioned cells. Granulocytes and macrophages with yellow pigment in their cytoplasm were evident at the periphery of the granuloma-like formations. These reactions, which never occurred in the control sponges or in those sponges which contained autologous-tissue extracts, apparently were due to local responses to heterologous chick embryo or extraembryonic antigens. There are several reports [139-143,161-170] demonstrating such antigenicity by serological and morphological methods. It is known that the development of antigens starts early in embryonic life.[144] It is probable that the necrotizing reaction following repeated resaturation of sponges with embryo extract, as described in "Results," as well as the granulomatous reaction described above, were both due to antigen-antibody reactions, descriptions of which are plentiful.[145-153] It has been shown [154,155] that the reaction is due to the antigen and antibody combining in the tissues. It appears reasonable that both these morphological events were manifestations of the Arthus phenomenon, which occurs readily in the rabbit.[156-160] It seems that with single exposures the resulting reactions were those of a subacute Arthus phenomenon, while an acute reaction was caused by multiple successive local injections into the sponge.

Summary

A method for the study of the influence of tissue extracts on the formation of granulation tissue in closed wounds is described. Ivalon sponges were used. The pores of control sponges were filled with equal parts of heparinized autologous plasma and Tyrode's solution, while those of experimental sponges were filled with equal parts of heparinized autologous plasma and a tissue extract. Three tissue extracts were used in different experiments. These were prepared by extracting homogenized chick embryos, autologous spleen, or autologous sterile post-traumatic granulation tissue with Tyrode's solution. Shortly after the plasma solutions had permeated the sponges, firm clots formed spontaneously throughout the interstices. The control and experimental sponges were then embedded on each side of the midline in intramuscular wounds in erector spinae muscles. Sixty rabbits were used. At periods of from 2 to 21 days after implantation the sponges and surrounding tissues were fixed in formalin. Histologic studies were then made of the granulation tissue growing into the pores of the sponges from the marginal wounded muscle.

Comparison of serial microscopic sections led to the following conclusions: At all comparable time intervals there were an increased number of fibroblasts and a deeper penetration of all components of granulation tissue into the pores of the experimental sponges. Also, there was an increased rate of maturation of collagenous fibrils in the sponges containing clots permeated with the tissue extracts. These differences were noted in the absence of any significant inflammatory-cell infiltration, except when chick embryo extract was used to dilute the plasma. The sterile inflammatory reaction in this instance was probably due to the presence of foreign protein from the chick embryo or adherent components of the egg.

Thus, it was possible to increase the rate of fibroblastic proliferation, capillary formation, and maturation of newly formed collagen in granulation tissue growing into a closed plasma clot system within a wound by addition of tissue extracts to that system.

Department of Pathology, Presbyterian-St. Luke's Hospital, 1753 W. Congress Pkwy. (Dr. Hass).

REFERENCES

1. Paschkis, K. E.: Growth-Promoting Factors in Tissues: A Review, Cancer Res. 18:981-991, 1958.

2. Swann, M. M.: The Control of Cell Division: II. Special Mechanisms, Cancer Res. 18:1118-1160, 1958.

3. Fischer, A.: The Nature of Growth-Promoting Substances in Embryonic Tissue Juice, Acta physiol. scandinav. 3:54-70, 1941.

4. Earle, W. R.: The Long Term Cultivation of Animal Tissues in Large Cultures, Fed. Proc. 17:967-974, 1958.

5. Sanford, K. K.; Earle, W. R., and Likely, G. D.: The Growth in Vitro of Isolated Tissue Cells, J. Nat. Cancer Inst. 9:229-246, 1946.

6. Schilling, J. A.; Favata, B. V., and Radakovich, M.: Studies of Fibroplasia in Wound Healing, Surg. Gynec. & Obst. 96:143-149, 1953.

7. Auerbach, E., and Doljanski, L.: The Effect of Cell Growth-Promoting Tissue Extracts on Healing of Experimental Cutaneous Wounds in Rats: I. Local Application, Brit. J. Exper. Path. 25:38-45, 1944.

8. van den Brenk, H. A. S.: Studies in Restorative Growth Processes in Mammalian Wound Healing, Brit. J. Surg. 43:525-550, 1956.

9. Grillo, H.; Watts, G. T., and Gross, J.: Studies in Wound Healing: I. Contraction and the Wound Contents, Ann. Surg. 148:145-152, 1958.

10. Watts, G.; Grillo, H., and Gross, J.: Studies in Wound Healing: II. The Role of Granulation Tissue in Contraction, Ann. Surg. 148:153-160, 1958.

11. du Noüy, P. L.: Mathematical Expression of the Curve Representing Cicatrization, J. Exper. Med. 24:451-470, 1916.

12. du Noüy, P. L.: Cicatrization of Wounds: X. A General Equation for the Law of Cicatrization of Surface Wounds, J. Exper. Med. 29:329-350, 1919.

13. Spain, K. C., and Loeb, L.: A Quantitative Analysis of the Influence of the Size of the Defect on Wound Healing in the Skin of the Guinea Pig, J. Exper. Med. 23:107-122, 1916.

14. Young, J. S.; Fisher, J. A., and Young, M.: Some Observations on the Healing of Experimental Wounds in the Skin of the Rabbit, J. Path. & Bact. 52:225-240, 1941.

15. Carrel, A., and Hartmann, A.: Cicatrization of Wounds: I. The Relationship Between the Size of a Wound and the Rate of Its Cicatrization, J. Exper. Med. 24:429-450, 1916.

16. Harvey, S. C.: Velocity of Growth of Fibroblasts in Healing Wound, Arch. Surg. 18:1227-1240, 1929.

17. Howes, E. L.; Sooy, J. W., and Harvey, S. C.: The Healing of Wounds as Determined by Their Tensile Strength, J.A.M.A. 92:42-45, 1929.

18. Sandblom, P.: The Tensile Strength of Healing Wounds: Experimental Study, Acta chir. scandinav. (Supp. 89) 90:1-108, 1944.

19. Charney, J.; Williamson, M. B., and Bernhart, F. W.: An Apparatus for the Determination of Tensile Strength of Healing Wounds, Science 105:396-397, 1947.

20. Dunphy, J. E.: Repair of Tissue After Injury, Ann. New York Acad. Sc. 73:426-437, 1958.

21. Dunphy, J. E., and Udupa, K. N.: Chemical and Histochemical Sequences in the Normal Healing of Wounds, New England J. Med. 253:847-851, 1955.

22. Williamson, M. B.: Protein Metabolism During Healing of Wounds, Clin. Chem. 2:1-15, 1956.

23. Taylor, F. W., and Ziperman, H. H.: Effect of Dicumarol on Wound Healing, Am. J. Surg. 80:113-116, 1950.

24. Barnes, C. T.; Karasic, J., and Amoroso, M. D.: Further Studies on the Rate of Healing of Human Skin Measured by the Electrical Wound Potential of Experimental Abrasions, Am. J. Surg. 82:720-726, 1951.

25. Hughes, A. F. W., and Dann, L.: Vascular Regeneration in Experimental Wounds and Burns, Brit. J. Exper. Path. 22:9-14, 1941.

26. Grindlay, J. H., and Waugh, J. M.: Plastic Sponge Which Acts as a Framework for Living Tissue, A.M.A. Arch. Surg. 63:288-297, 1951.

27. Bouchek, R. J., and Noble, N. L.: Connective Tissue: A Technic for Its Isolation and Study, A.M.A. Arch. Path. 59:553-558, 1955.

28. Davila, J. C.; Schultz, D., and Aronstam, E. M.: Suitability of Ivalon Sponge Tubes for Replacing Vascular Segments, U.S. Armed Forces M.J. 8:1452-1466, 1957.

29. Gale, J. W.; Curreri, A. R.; Young, W. P., and Dickie, H. A.: Plastic Sponge Prosthesis Following Resection in Pulmonary Tuberculosis, J. Thoracic Surg. 24:587-610, 1952.

30. Bing, J.: The Tissue Reaction to Implanted Plastics, Acta path. et microbiol. scandinav., Supp. 105, pp. 16-26, 1955.

31. Edwards, L. C.; Pernokas, L. N., and Dunphy, J. E.: The Use of a Plastic Sponge to Sample Regenerating Tissue in Healing Wounds, Surg. Gynec. & Obst. 105:303-309, 1957.

32. LeVeen, H. H., and Barberio, J. R.: Tissue Reaction to Plastics Used in Surgery, with Special Reference to Teflon, Ann. Surg. 129:74-84, 1949.

33. Martinez, N. S.; Dahl, E. V., and Grindlay, J. H.: Early Tissue Response to Polyvinyl Sponge Aortic Grafts: Experimental Study, Surgery 42: 1002-1013, 1957.

34. Harrison, J. H.: A Teflon Weave for Replacing Tissue Defects, Surg. Gynec. & Obst. 104: 584-590, 1957.

35. Carrel, A., and Burrows, M. T.: Cultivation of Adult Tissues and Organs Outside of the Body, J.A.M.A. 55:1379-1380, 1910.

36. Carrel, A., and Burrows, M. T.: Cultivation of Sarcoma Outside of the Body: A Second Note, J.A.M.A. 55:1554, 1910.

37. Carrell, A., and Burrows, M. T.: Artificial Stimulation and Inhibition of the Growth of Nor-

mal and Sarcomatous Tissues, J.A.M.A. 56:32-33, 1911.

38. Burrows, M. T.: The Cultivation of Tissues of the Chick Embryo Outside the Body, J.A.M.A. 55:2057-2058, 1910.

39. Carrel, A.: Rejuvenation of Cultures of Tissues, J.A.M.A. 57:1611, 1911.

40. Carrel, A.: On the Permanent Life of Tissues Outside of the Organism, J. Exper. Med. 15:516-528, 1912.

41. Carrel, A.: Artificial Activation of Growth of Connective Tissue, J. Exper. Med. 17:14-19, 1913.

42. Carrel, A., and Ebeling, H.: Pure Cultures of Large Mononuclear Leucocytes, J. Exper. Med. 36:365-377, 1922.

43. Carrel, A., and Lindbergh, C.: The Culture of Organs, Paul B. Hoeber, Inc. (Medical Book Department of Harper & Brothers), 1938.

44. Hass, G. M., and McDonald, F.: Studies of Collagen: Production of Collagen in Vitro Under Variable Experimental Conditions, Am. J. Path. 16:525-548, 1940.

45. Young, J. S.; Cruickshank, A. H., and Martin, W. J.: An Experimental Study of the Healing of Wounds, with Special Reference to the Action of Heart Extract Powder (Doljanski), J. Path. & Bact. 58:63-74, 1946.

46. Lillie, R. D.: Histopathologic Technic and Practical Histochemistry, Ed. 2, New York, The Blakiston Company (Division of McGraw-Hill Book Company, Inc.), 1954.

47. Manual of Histologic and Special Staining Technics, Washington, D.C., Armed Forces Institute of Pathology, 1957.

48. Howes, E. L.: The Connective Tissues in Wound Healing, in Connective Tissue in Health and Disease, edited by G. Asboe-Hansen, Copenhagen, Ejnar Munksgaard Forlag, 1954, pp. 159-177.

49. Banfield, W. G.: Aging of Connective Tissue, in Connective Tissue in Health and Disease, edited by G. Asboe-Hansen, Copenhagen, Ejnar Munksgaard Forlag, 1954, pp. 151-158.

50. Jackson, D. S.: Some Biochemical Aspects of Fibrogenesis and Wound Healing, New England J. Med. 259:814-820, 1958.

51. Lattes, R.; Martin, J. R., and Ragan, C.: Suppression of Cortisone Effect on Repair in the Presence of Local Bacterial Infection, Am. J. Path. 30:901-912, 1954.

52. Prudden, J. F.; Nisihara, G., and Baker, L.: The Acceleration of Wound Healing with Cartilage: I, Surg. Gynec. & Obst. 105:283-286, 1957.

53. Loeb, L.: A Comparative Study of the Mechanism of Wound Healing, J.M. Res. 41:247-281, 1920.

54. Barth, L. G.: Developmental Physiology, Ann. Rev. Physiol. 19:41-58, 1957.

55. Eremeer, N. S.: Peculiarities of Reparatory Regeneration of Skin After Denervation, Arkh. anat. gist. i embriol. 34:88-96, 1957.

56. Kiss, F., and Lang, A.: Innervation und Entwicklung der kollagen Fasern, Anat. Anz. 104: 401-411, 1957.

57. Clark, E. R.; Hitschler, W. T.; Smith, H. T.; Rex, R. O., and Smith, J. H.: General Observations of New Blood Vessels into Standardized Chambers in the Rabbit's Ear, and Subsequent Changes in the Newly Grown Vessels over a Period of Months, Anat. Rec. 50:129-168, 1931.

58. Stearns, M. L.: Studies on the Development of Connective Tissue in Transparent Chambers in the Rabbit's Ear: I, Am. J. Anat. 66:133-176, 1940.

59. Stearns, M. L.: Studies on the Development of Connective Tissue in Transparent Chambers of the Rabbit's Ear: II, Am. J. Anat. 67:55-97, 1940.

60. Whipple, A. O.: The Critical Latent or Lag Period in the Healing of Wounds, Ann. Surg. 112:481-488, 1940.

61. Banfield, W. G.: Width and Length of Collagen Fibrils During the Development of Human Skin, in Granulation Tissue, and in Vein of Adult Animals, J. Gerontol. 10:13-17, 1955.

62. Allgöwer, M.: The Cellular Basis of Wound Repair, Springfield, Ill., Charles C Thomas, Publisher, 1956.

63. Brandaleone, H., and Papper, E.: The Effect of the Local and Oral Administration of Cod Liver Oil on the Rate of Wound Healing in Vitamin A-Deficient and Normal Rats, Ann. Surg. 114:791-798, 1941.

64. Berenblum, I.: Irritation and Carcinogenesis, Arch. Path. 38:233-244, 1944.

65. Auerbach, E., and Doljanski, L.: Effect of Cell Growth Activating Extracts, Parenterally Applied, on Experimental Skin Wounds, Proc. Soc. Exper. Biol. & Med. 58:111-112, 1945.

66. Teir, H.; Putkonen, T., and Kilijunen, A.: Effects of Parenterally Applied Skin Extracts on Wound Healing in the White Rat, Ann. chir. et gynæc. Fenniae 40:51-60, 1951.

67. Kelly, L. S., and Jones, H. B.: Influence of Homologous Tissue Factors on DNA Turnover and Radiation Protection, Am. J. Physiol. 172: 575-578, 1953.

68. Kaufman, L.: Expériences sur l'effet des injections d'extraits de sérum d'animaux âgés et d'extraits d'embrynos sur la croissance des souris, Compt. rend. Soc. biol. 110:1094-1096, 1932.

69. Carrel, A.: The Treatment of Wounds, J.A.M.A. 55:2148-2150, 1910.

70. Carrell, A.: Cicatrization of Wounds: XII. Factors Initiating Regeneration, J. Exper. Med. 34:425-434, 1921.

71. Carrel, A.: The Process of Wound Healing, Proc. Inst. Med. Chicago 8:62-66, 1930.

72. Nakamura, T.: Action des extraits d'embryons sur la cicatrisation et la régénération, Compt. rend. Soc. biol. 104:191-192, 1930.

73. Nielsen, E.: Effect of Animal Growth Substances on Course of Wound Healing, Ugesk. læger 101:1071-1075, 1939.

74. Nielsen, N. B.: Clinical Experiences with Growth Promoting Fractions of Embryonic Juice, Nord. med. 14:1553-1555, 1942.

75. Wallich, R.: Contribution à l'étude de la culture des tissus in vivo dans les phénomènes de cicatrisation des plaies superficielles, Compt. rend. Soc. biol. 95:1480-1482, 1926.

76. Morosov, D. B., and Striganova, A. R.: L'action stimulante des extraits embryonaires sur la cicatrisation des plaies chez les mammifères, J. physiol. et path. gén. 32:1148-1152, 1938.

77. Roulet, F.: Stimulating Action of Embryonic Juice on the Burgeoning of Experimental Skin Wounds in the Guinea Pig, Compt. rend. Soc. biol. 95:390-392, 1926.

78. Lorin-Epstein, M. J.: Über einige allgemeine Faktoren der Wiederherstellungsprozesse und ihre Bedeutung für die chirurgische Pathologie, Arch. klin. Chir. 144:632-666, 1927.

79. Carnot, P., and Terris, E.: Cicatrization of Skin Wounds by Embryonic Extracts or Regenerated Skin, Compt. rend. Soc. biol. 95:655-657, 1926.

80. Kiaer, S.: Biologische Beobachtung über Wundheilung (Beschleunigung mit Hühnerembryonalsaft und Proteosen), Arch. klin. Chir. 149: 146, 1928.

81. Kiaer, S.: Biologische Beobachtung über Wundheilung: Über die Möglichkeit des Wundheilungsprozesses mittels Hühner-Embryonalsaft und Proteosen zur Beschleunigung), Arch. klin. Chir. 148:156-182, 1927.

82. Dann, L.; Glücksmann, A., and Tansley, K.: Healing of Wounds Treated with Epicutan, Brit. J. Exper. Path. 22:70-75, 1941.

83. Doljanski, L., and Auerbach, E.: Effect of Cell Growth Activating Tissue Extracts, Locally Applied on Experimental Skin Wounds, Proc. Soc. Exper. Biol. & Med. 55:112-113, 1945.

84. Doljanski, L.; Hoffman, R. S., and Tanenbaum, E.: Effects of Extracts of Heterologous Adult Tissue on Cell Growth in Vitro and Their Use in Wound Healing, Nature, London 150:23-24, 1942.

85. McJunkin, F., and Matsui, T.: Effect of Homologous Macerated Skin on the Regeneration of Epidermis, Arch. Path. 12:794-801, 1931.

86. Hoffman, R. S., and Dingwall, J. A., III: An Experimental and Clinical Study on the Use of Adult Animal Tissue Extract in the Acceleration of Wound Healing, Surg. Gynec. & Obst. 79:103-108, 1944.

87. Mandl, F.: Gewebsextrakte und Pulver zur Heilung von "indolenten" Wunden und Geschwüren, Ars med. 36:430-435, 1946.

88. Kerr, A. B., and Werner, H.: The Clinical Value of a Growth-Promoting Substance in Treatment of Indolent Wounds, Brit. J. Surg. 32:281-287, 1945.

89. Kerr, A. B., and Werner, H.: Further Observations on the Clinical Value of a Growth-Promoting Substance in Wound Treatment, Brit. J. Surg. 32:518-524, 1945.

90. Seldon, T. H.; Lundy, J. S., and Adams, R. C.: Stimulation of Wound Healing: New Use of Powdered Blood Cells, Anesthesiology 5:566-573, 1944.

91. Dvorak, H. J., and Byram, J. W.: Effect of Addition of Macerated Tissues on Healing of Granulating Wounds, Proc. Soc. Exper. Biol. & Med. 27:967-969, 1930.

92. Edwards, R.; Maganini, H.; Sarmenta, S., and Hass, G. M.: Comparative Stimulation of Growth and Differentiation of Fibroblasts by Tissue Extracts in Vivo and in Vitro, Fed. Proc. 17:435, 1958; abstract 1709.

93. Day, T. D.: The Localizing Action of Fibrinous Exudates, Brit. J. Exper. Path. 55:315-321, 1954.

94. Menkin, V.: Biochemical Mechanisms in Inflammation, Ed. 2, Publication No. 288, American Lecture Series, monograph in Bannerston Division of American Lectures in Dentistry, edited by E. J. Ryan, Springfield, Ill., Charles C Thomas, Publisher, 1956.

95. Porter, K. R., and Hawn, C. V.: Sequences in the Formation of Clots from Purified Bovine Fibrinogen: A Study with the Electron Microscope, J. Exper. Med. 90:225-231, 1949.

96. Dann, L.; Glücksmann, A., and Tansley, K.: Experimental Wounds Treated with Cod-Liver Oil and Related Substances, Lancet 1:95-98, 1942.

97. Sutton, L. E.: Thioglycerol—a More Stable Sulphhydril for Use in the Healing of Wounds, J.A.M.A. 104:2168-2171, 1935.

98. Reimann, S. P.: Use and Reasons for Use of Thiocresol to Stimulate Wound Healing, J.A.M.A. 94:1369-1371, 1930.

99. Localio, S. A.; Morgan, M. E., and Hinton, J. W.: Biological Chemistry of Wound Healing: I. Effect of dl-Methionine on Healing of Wounds in Protein-Depleted Animals, Surg. Gynec. & Obst. 86:582-590, 1948.

100. Brunsting, L. A., and Simonsen, D. G.: Cutaneous Ulcers Treated by the Sulfhydryl Containing Amino Acid Cysteine, J.A.M.A. 101:1937-1940, 1937.

101. Hammett, F. S., and Reimann, S. P.: Cell Proliferation Response to Sulfhydryl in Mammals, J. Exper. Med. 50:445-448, 1929.

102. Smith, L. W., and Livingston, A. E.: Chlorophyll: An Experimental Study of Its Water

Soluble Derivatives in Wound Healing, Am. J. Surg. 62:358-369, 1943.

103. Moss, H.; Morrow, B. A.; Long, R. C., and Ravdin, I. S.: Effectiveness of Chloresium in Wound Healing and Deodorant Effects, J.A.M.A. 140:1336-1339, 1949.

104. Hermannsdorfer, A.: Über den Einfluss der Nahrung auf die Pufferkapazität des Blutes und den Heilverlauf und Keimgehalt granulierender Wunden, Deutsche Ztschr. Chir. 200:534-583, 1927.

105. Apperly, F. L., and Cary, M. K.: Epithelization of Experimental Wounds, Arch. Surg. 49: 327-330, 1944.

106. Pirani, C. L.; Stepto, R. C., and Sutherland, K.: Desoxycorticosterone Acetate and Wound Healing, J. Exper. Med. 93:217-228, 1951.

107. Taubenhaus, M., and Amromin, G. D.: Influence of Steroid Hormones on Granulation Tissue, Endocrinology 44:359-367, 1949.

108. Sellers, E. A.; You, S. S., and You, R. W.: The Influence of Adrenal Cortex and Thyroid on the Loss of Nitrogen in Urine After Experimental Burns, Endocrinology 47:148-155, 1950.

109. Taubenhaus, M.; Taylor, B., and Morton, J. V.: Hormonal Interaction in the Regulation of Granulation Tissue Formation, Endocrinology 51: 183-191, 1952.

110. Gribble, M. de G., and Peters, R. A.: Influence of Thyroidectomy on Post-Burn N Loss in Rats, Quart. J. Exper. Physiol. 36:119-126, 1951.

111. Taubenhaus, M., and Amromin, G. D.: The Effects of the Hypophysis, Thyroid, Sex Steroids, and the Adrenal Cortex upon Granulation Tissue, J. Lab. & Clin. Med. 36:7-18, 1950.

112. Scow, R. O.: Effect of Growth Hormone on Muscle and Skin Collagen in Neonatal Thyroidectomized Rats, Endocrinology 49:641-646, 1951.

113. Wase, A. W.; Eichel, H. J., and Replinger, E.: Relation of Thyroid Status to Nitrogen Excretion Following Exposure to Thermal Radiation, Proc. Soc. Exper. Biol. & Med. 84:152-154, 1953.

114. Boas, N. F., and Foley, T. B.: Regulation of Connective Tissue Hexosamine Levels by the Anterior Pituitary and Thyroid Glands, Proc. Soc. Exper. Biol. & Med. 87:89-91, 1954.

115. Baker, B. L.: The Connective Tissue Reaction Around Implanted Pellets of Steroid Hormones, Anat. Rec. 119:529-539, 1954.

116. Iversen, K.: Hormonal Influence on Connective Tissue, in Connective Tissue in Health and Disease, edited by G. Asboe-Hansen, Copenhagen, Ejnar Munksgaard Forlag, 1954.

117. Snelser, G. K., and Ozanics, V. J.: Effects of Thyroid Hormone on Mitosis and Regeneration in Corneal Epithelium, J. Cell. & Comp. Physiol. 43:107-117, 1954.

118. Moltke, E.: Wound Healing Influenced by Thyroxine and Thyrotropic Hormone: A Tensiometric Study, Proc. Soc. Exper. Biol. & Med. 88: 596-599, 1955.

119. Selye, H.: Anticortisol Action of Aldosterone, Science 121:368-369, 1955.

120. Blomqvist, K.: Growth Stimulation in the Liver and Tumor Development Following Intraperitoneal Injections of Liver Homogenates in the Rat, Acta path. et microbiol. scandinav., Supp. 131, pp. 1-65, 1957.

121. Brues, A. M.; Subbarow, Y.; Jackson, E. B., and Aub, J. C.: Growth Inhibition by Substances in Liver, J. Exper. Med. 71:423-438, 1940.

122. Cantarow, A.; Paschkis, K. E., and Stasney, C.: Tumor Growth in Partially Hepatectomized Rats, Fed. Proc. 14:25, 1955.

123. Christensen, B. G., and Jacobson, E.: Studies on Liver Regeneration, Acta med. scandinav., Supp. 234, pp. 103-108, 1950.

124. Udupa, K. N.; Woessner, J. F., and Dunphy, J. E.: The Effect of Methionine on the Production of Mucopolysaccharides and Collagen in Healing Wounds of Protein-Depleted Animals, Surg. Gynec. & Obst. 102:639-645, 1956.

125. Thompson, W. D.; Ravdin, I. S., and Frank, I. L.: Effect of Hypoproteinemia on Wound Disruption, Arch. Surg. 36:500-508, 1938.

126. Rhoads, J. E.; Fliegelman, M. T., and Panzer, L. M.: The Mechanism of Delayed Wound Healing in the Presence of Hypoproteinemia, J.A.M.A. 118:21-25, 1942.

127. Varco, R. L.: Nutritional Preparation for Substandard Risk Patients, Surg. Gynec. & Obst. 84:611-618, 1947.

128. Lowry, K. F., and Curtis, G. M.: Pertinent Factors Influencing Repair in Traumatic Wounds, Am. J. Surg. 78:781-792, 1949.

129. Howes, E. L.; Briggs, H.; Shea, R., and Harvey, S. C.: Effect of Complete and Partial Starvation on the Rate of Fibroplasia in the Healing Wound, Arch. Surg. 27:846-858, 1933.

130. Harvey, S. C., and Howes, E. L.: Effect of High Protein Diet on the Velocity of Growth of Fibroblasts in the Healing Wound, Ann. Surg. 91:641-650, 1930.

131. Williamson, M. B.; McCarthy, T. H., and Fromm, H. J.: Relation of Protein Nutrition to the Healing of Experimental Wounds, Proc. Soc. Exper. Biol. & Med. 77:302-305, 1951.

132. Wolbach, S. B., and Howe, P.: Intercellular Substances in Experimental Scorbutus, Arch. Path. & Lab. Med. 1:1-24, 1926.

133. Wolbach, S. B.: Controlled Formation of Collagen and Reticulin: A Study of the Source of Intercellular Substance in Recovery from Experimental Scorbutus, Am. J. Path. 9:689-699, 1933.

134. Wolbach, S. B., and Bessey, O. A.: Tissue Changes in Vitamin Deficiencies, Physiol. Rev. 22:233-289, 1942.

135. Lanman, T. H., and Ingalls, T. H.: Vitamin C Deficiency in Wound Healing: Experimental and Clinical Study, Ann. Surg. 105:616-625, 1937.

136. Lund, C. C., and Crandon, J. H.: Ascorbic Acid and Human Wound Healing, Ann. Surg. 115:776-790, 1941.

137. Cameron, G. R.: Pathology of the Cell, London, Oliver & Boyd, Ltd., 1952.

138. Gillman, J., and Penn, J.: Studies on the Repair of Cutaneous Wounds, Med. Proc. 3:93-186, 1956.

139. Nace, G. W.: Development in the Presence of Antibodies, Ann. New York Acad. Sc. 60:1038-1055, 1955.

140. Cooper, R. S.: Adult Antigens (or Specific Combining Groups) in the Egg, Embryo, and Larva of the Frog, J. Exper. Zool. 101:143-172, 1946.

141. Schechtman, A. M.: Antigens of Early Developmental Stages of the Chick, J. Exper. Zool. 105:329-348, 1946.

142. ten Cate, G., and Doorennalen, W. G.: Analysis of the Development of the Eye-Lens in Chicken and Frog by Means of the Precipitation Reaction, Koninkl. nederl. Akad. Wetenschap 53:3-18, 1950.

143. Woerdeman, N. W.: Serological Methods in the Study of Morphogenesis, Arch. nederl. zool. (Supp. 1) 10:144-159, 1953.

144. Simonsen, M: The Impact on the Developing Embryo and Newborn Animal of Adult Homologous Cells, Acta path. et microbiol. scandinav. 40:480-500, 1957.

145. Loeb, L.: The Biological Basis of Individuality, Springfield, Ill., Charles C Thomas, Publisher, 1945.

146. Sabin, F. R.; Doan, C. A., and Forkner, C. E.: Studies on Tuberculosis, J. Exper. Med. (Supp. 3) 52:1-152, 1930.

147. Medawar, P. B.: The Behaviour and Fate of Skin Autografts and Skin Homografts in Rabbits (Report of War Wounds Committee of Medical Research Council), J. Anat. 78:176-199, 1944.

148. Medawar, P. B.: A Second Study of the Behaviour of Skin Homografts in Rabbits, J. Anat. 79:157-176, 1945.

149. Ehrich, W. E.; Seifter, J., and Forman, C.: Experimental Serum Disease: A Pathogenic Study, J. Exper. Med. 89:23-36, 1949.

150. Fagraeus, A.: Antibody Production in Relation to the Development of Plasma Cells, Acta med. scandinav. (Supp. 204) 130:3-122, 1948.

151. Darcy, D. A.: Plasma Cells in the Reaction Against Rabbit Tissue Homografts, Nature, London 163:98-99, 1949.

152. Goddard, J. W.: Granuloma, a Characteristic "Qualitative" Change in Focal Anaphylactic Inflammation, Am. J. Path. 23:943-965, 1947.

153. Goldgraber, M. B., and Kirsner, J. B.: Granulomatous Lesions—an Expression of a Hypersensitive State: An Experimental Study, A.M.A. Arch. Path. 66:618-634, 1958.

154. Opie, E. L.: The Fate of Antigen (Protein) in an Animal Immunized Against It, J. Exper. Med. 39:659-675, 1924.

155. Opie, E. L.: Inflammation and Immunity, J. Immunol. 17:329-342, 1929.

156. Boyd, W. C.: Fundamentals of Immunology, New York, Interscience Publishers, 1956.

157. Ingalls, M. S.: A Contribution to the Nature and Origin of Natural Agglutinins, J. Immunol. 33:123-148, 1937.

159. Benacerraf, B., and Kabat, E. A.: A Quantitative Study of the Arthus Phenomenon Induced Passively in the Guinea Pig, J. Immunol. 64:1-19, 1950.

158. Germuth, F. G., Jr.: A Comparative Histologic and Immunologic Study in Rabbits of Induced Hypersensitivity of the Serum Sickness Type, J. Exper. Med. 97:257-282, 1953.

160. Germuth, F. G., Jr.; Pace, M. D., and Trippet, J. C.: Comparative Histologic and Immunologic Study in Rabbits of Induced Hypersensitivity of the Serum Sickness Type, J. Exper. Med. 101:135-150, 1955.

161. Wells, H. G.: Studies on the Chemistry of Anaphylaxis: II, J. Infect. Dis. 6:506-522, 1909.

162. Wells, H. G.: Studies on the Chemistry of Anaphylaxis: III. Experiments with Isolated Proteins, Specially Those of the Hen's Egg, J. Infect. Dis. 9:147-171, 1911.

163. Emmerich, E. Z.: Untersuchungen mit eigelbantiseren zugleich ein Beitrag zu den Beziehungen der verschiedenen Eigelbarten zueinander, Ztschr. immunol. Forsch. 17:299-304, 1913.

164. Seng, H.: Untersuchungen mit Hühnereigelb-Antiserum, Ztschr. Immunol. Forsch. 20:355-366, 1913.

165. Jukes, T. H., and Kay, H. D.: Egg-Yolk Proteins, J. Nutrition 5:81-101, 1932.

166. Jukes, T. H., and Kay, H. D.: The Immunological Behaviour of the Second Protein (Livetin) of Hen's Egg Yolk, J. Exper. Med. 56:469-482, 1932.

167. Roepke, R. R., and Bushnell, L. D.: A Serological Comparison of the Phosphoprotein of the Serum of the Laying Hen and the Vitelline of Egg Yolk, J. Immunol. 30:109-113, 1936.

168. Francis, G. E., and Wormall, A.: The Use of Radioactive Isotopes in Immunological Investigations: I. Percipitin Reaction with Ovovitellin Containing P^{32}, Biochem. J. 42:469-474, 1948.

169. Chargaff, E.: A Study of Lipoproteins, J. Biol. Chem. 142:491-504, 1942.

170. Chargaff, E.: The Formation of Phosphorus Compounds in Egg Yolk, J. Biol. Chem. 142:505-512, 1942.

Liver Dehydrogenase Activity in Chronic Alcoholism

A Compartive Histochemical Study

SAMUEL W. FRENCH, M.D., San Francisco

The present study is concerned with the pathogenesis of hepatocellular damage in chronic alcoholic patients. Various contributing factors to hepatocellular damage in these patients have been implicated, such as malnutrition, chronic alcohol intoxication, and ischemia.[1] However, the exact mechanism or mechanisms of injury remain unproved.

In the present investigation livers of chronic alcoholic patients were studied by histochemical assay of diphosphopyridine nucleotide dehydrogenase (DPNHD) and succinic dehydrogenase system (SHD). Livers of patients with a variety of other diseases but without a history of chronic alcoholism were similarly studied for comparison.

It was thought likely that distinctive differences in patterns of enzyme-activity staining would be revealed by means of these histochemical techniques. Supporting this view, cellular SHD and DPNHD have responded differently to some toxins,[2] changes in oxygen tension,[3,4] and riboflavin deficiency.[5,6] Differences in the activity of these enzymes have also been observed in neoplasia[7] and regeneration.[8]

Material and Methods

Liver was obtained from 25 alcoholics and 24 nonalcoholics by laparotomy or needle biopsy or at autopsy. The patients with a history of alcoholism ranged from 33 to 66 years in age; 12 were women and 13 were men; 2 were Negro and 23 were white. The patients without a history of alcoholism ranged from 14 to 80 years old; 9 were women and 15 were men; 4 were Negro, 2 were Oriental, and 18 were white.

Tables 1 and 2 list the cases studied. The patients listed in Table 2 gave a history of either chronic alcoholism or excessive drinking with the exception of Case 21. Twenty-one of the cases in Table 2 had objective evidence of organ damage resulting from alcoholism. The patients listed in Table 1 did not have a history of alcoholism.

For this study the stages of liver disease in the alcoholics were divided into three main categories: fatty liver, active cirrhosis, and inactive cirrhosis. In the fatty livers over 50% of the cells were distended by large fat spheres without excess fibrosis or lobular distortion. In active cirrhosis the livers contained fat-laden liver cells, "alcoholic hyalin" bodies, neutrophil infiltrate, and evidence of bile stasis in addition to cirrhosis. In inactive cirrhosis there were fibrosis and regenerative nodule formation without evidence of hepatocellular degenerative changes.

The histochemical methods of Farber et al.[9,10] were modified by replacing blue tetrazolium with nitro-BT.[11] The medium for the succinic dehydrogenase system contained 0.3 ml. sodium succinate (0.5 M), 1 ml. potassium phosphate buffer (0.1 M, pH 7.4), 0.3 ml. calcium chloride (0.004 M), 0.15 ml. sodium bicarbonate (0.6 M, freshly prepared), 0.3 ml. nitro-BT (5 mg. per milliliter) 0.05 ml. phenazine methosulfate (1 mg. per milliliter), and 0.9 ml. of distilled water. The medium for diphosphopyridine nucleotide dehydrogenase contained 0.3 ml. sodium *l*-malate (0.5 M), 0.2 ml. ethanol (1.09 M), 0.5 ml. sodium L-glutamate (0.5 M), 0.8 ml. potassium phosphate buffer (0.1 M, pH 7.4), 0.02 ml. alcohol dehydrogenase, 0.2 ml. diphosphopyridine nucleotide (5 mg. per milliliter), 0.2 ml. semicarbazide (0.1 M), 0.3 ml. nitro-BT (5 mg. per milliliter) 0.05 ml. Azure I (mixture of azure A and azure B) (1 mg. per milliliter), and 0.43 ml. distilled water. All substrate solutions were neutralized to pH 7.4. Chemicals were obtained from sources used by the authors cited above, with the exception of phenazine methosulfate, which was made according to the method of Kehrmann and Havas.[12]

Submitted for publication May 19, 1959.

Department of Pathology, University of California Medical Center.

This work was supported in part by U.S. Public Health Service Grant CRTY 5029 and the Committee on Research, University of California School of Medicine.

TABLE 1.—*Enzyme Activity in Livers of Patients Without a History of Alcoholism*

Case	PM * Hours	NB †	SB ‡	Morphologic Findings	Enzyme Activity: DPNHD §	Enzyme Activity: SHD ‖
1,2 ¶		x		Normal	Normal	Normal
3			x	Normal	Normal	Normal
4			x	Normal and hepatoma	Normal	Normal
5	4			Normal	Normal	Normal
6	6			Normal	Normal	Normal
7	8			Normal	Normal	Normal
8	8			Normal	Normal	Reduced focally
9		x		Portal fibrosis	Normal	Normal
10			x	Cholangitis	Normal	Reduced centrally
11			x	Bile stasis	Reduced centrally	Reduced centrally
12	12			Portal fibrosis	Normal	Reduced centrally
13	6.5			Central congestion	Absent focally	Absent focally
14	22			Central congestion	Reduced centrally	Greatly reduced centrally
15	22			Central congestion	Greatly reduced in necrotic zones	Greatly reduced in necrotic zones
16	22			Central necrosis	Absent in necrotic zones	Absent in necrotic zones
17	4			Central necrosis	Absent in necrotic zones	Absent in necrotic zones
18	10			Early central necrosis	Absent in necrotic zones	Reduced centrally
19,20 ¶		x		Viral hepatitis	Normal	Normal
21		x		Viral hepatitis	Unstained cells centrally	Unstained cells centrally
22	8			Viral hepatitis with massive necrosis	Greatly reduced	Greatly reduced
23	16			Focal periportal fat	Normal	Normal
24			x	Central fat	Normal	Reduced periportally

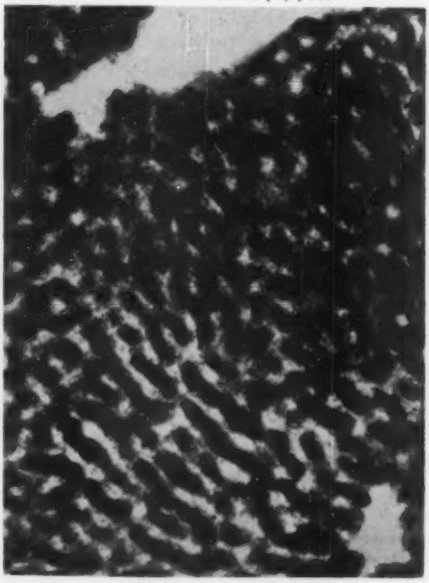
* Post mortem. † Needle biopsy. ‡ Surgical wedge biopsy. § Diphosphopyridine nucleotide dehydrogenase. ‖ Succinic dehydrogenase system. ¶ Cases considered together because of identical findings.

Tissues were removed and promptly placed in a freezing compartment, where they were stored at —10C. The tissues were stored for periods ranging from a few minutes to three days before the histochemical stains were applied. The staining quality of tissue stored at this temperature did not change for six days or longer. Tissues were cut 15μ thick on a freezing microtome, floated onto round cover slips in saline, and transferred to 20 cc. beakers containing the medium. The tissue slices were incubated in the medium for 2 to 15 minutes at 37 C in a Dubnoff metabolic shaker. The atmosphere above the beakers was flushed continuously with N_2. The reaction was stopped with 10% buffered neutral formalin, and the slices were mounted with Paragon frozen-section-mounting medium. The adjacent liver tissue was fixed in buffered neutral formalin, mounted in paraffin, and stained with hematoxylin and eosin.

Results

Conditions Other Than Alcoholism (Table 1).—The sections of morphologically normal livers for biopsy or autopsy consistently stained uniformly for DPNHD. In one of the normal livers obtained post mortem, staining for SHD was reduced focally in hepatic parenchyma, but it was otherwise uniform throughout the lobules of histologically normal livers (Fig. 1). The bile duct epithelium and smooth muscle cells of blood vessels stained well for DPNHD but

Fig. 1.—Normal liver (nonalcoholic). Liver cells stain uniformly throughout the lobule. The portal tract above shows no enzyme activity. There is a central vein below. SHD; × 50.



poorly for SHD (Fig. 2). Kupffer's cells and sinusoidal lining cells and portal connective tissue cells stained fairly well for DPNHD (Figs. 2 and 4). Age and sex did not influence the staining pattern. Since most of the patients were white, the influence of race on the staining pattern could not be evaluated.

In some livers showing evidence of passive congestion at autopsy, DPNHD and SHD activity were reduced in the centrilobular zone. In one case of congested liver there were rare pericentral discrete groups of unstained liver cells in the absence of necrotic changes. Sinusoidal lining cells in these zones stained well (Fig. 4). When central necrosis was evident in sections stained with hematoxylin and eosin, the same pattern of central nonstaining for DPNHD and SHD activity was found (Fig. 3). Sinusoidal lining cells retained their activity in these necrotic zones.

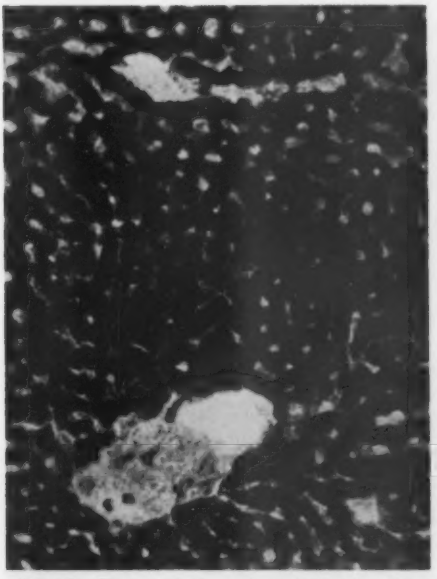


Fig. 2.—Congested liver (nonalcoholic). Liver cells and bile ducts stain uniformly. Portal structures show enzyme activity. There is a portal tract below and a central vein above. DPNHD; × 50.

Bile stasis, indicated by bile plugs in canaliculi or by rounded masses of bile

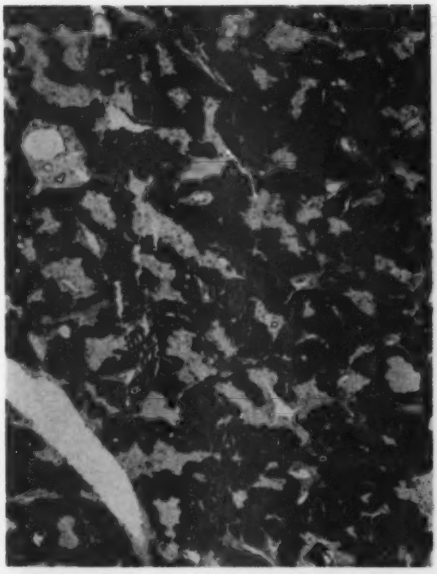
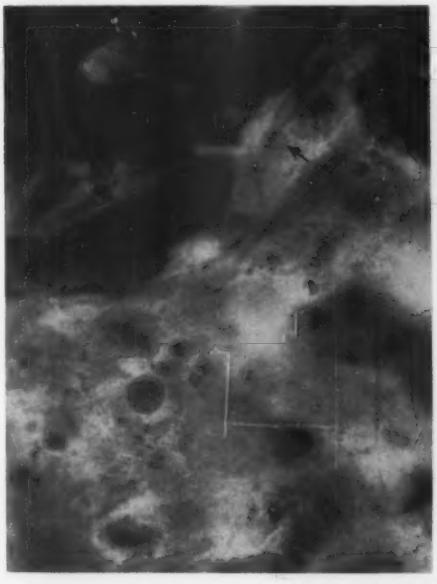


Fig. 3.—Central necrosis (nonalcoholic). Note discrete zones of nonstaining liver cells in the center of the lobules. There is a hepatic vein in the lower left corner. DPNHD; × 7.9.

Fig. 4.—Congested liver (nonalcoholic). The boundary of liver cells, showing absence of enzyme activity, is well demarcated from surrounding normal liver. Stained cell indicated by arrow is a sinusoidal-lining cell. DPNHD; × 537.



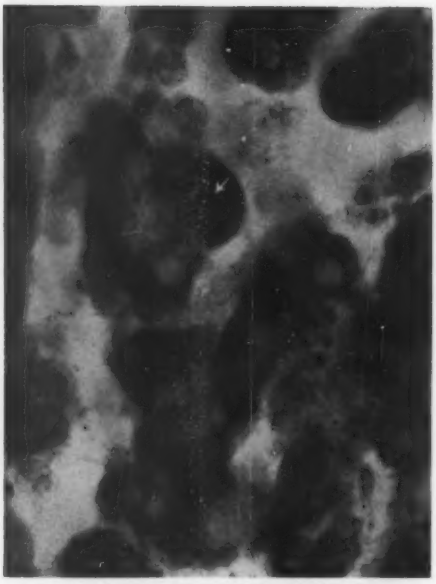


Fig. 5.—Congested liver (nonalcoholic). Note masses of bile and lipofuchsin not associated with reduced enzyme activity of liver cells. DPNHD; × 500.

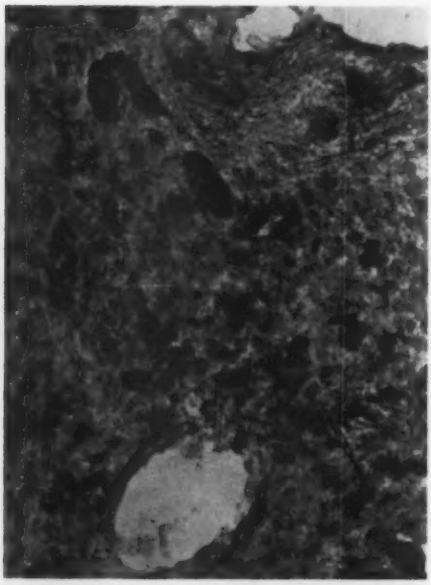


Fig. 6.—Fatal viral hepatitis (nonalcoholic). A few periportal cholangioles show enzyme activity. All liver cells are necrotic and show greatly reduced activity. Dark masses are globules of brown pigment. Portal tract is located above and central vein below. DPNHD; × 125.

Fig. 7.—Diffusely fatty liver (alcoholic). There is absence or reduction of enzyme activity in central zones. The borders of these zones are poorly defined. DPNHD; × 6.6.

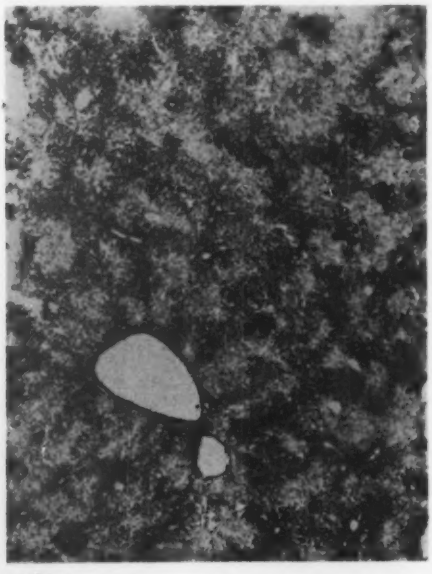
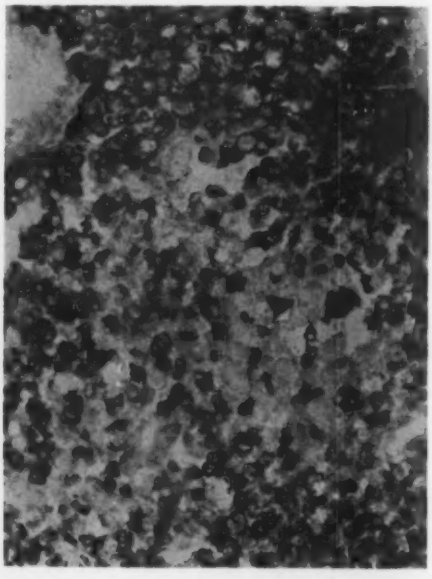


Fig. 8.—Diffusely fatty liver (alcoholic). Many fat-laden liver cells showing no activity are interspersed between cells showing normal activity. There is a portal tract in the upper right corner. DPNHD; × 71.



within liver cells, was not associated with reduced staining of the involved cells (Fig. 5).

One section of liver from a patient with viral hepatitis did not stain for SHD and DPNHD in the central zones. Liver cells in a case of fulminating hepatitis with massive liver necrosis took almost no stain (Fig. 6). In this case a few tubule-like groups of liver cells near portal tracts retained evidence of activity.

In the one fatty liver obtained post mortem, the fat was deposited only in one focus, 3 cm. in diameter, next to the falciform ligament. Here the distribution of the fat was predominantly periportal. SHD staining and DPNHD staining were uniform throughout the lobule. In one biopsied liver containing centrilobular fat, SHD staining was very faint peripherally. The fat-laden centrally located cells stained well (Fig. 11).

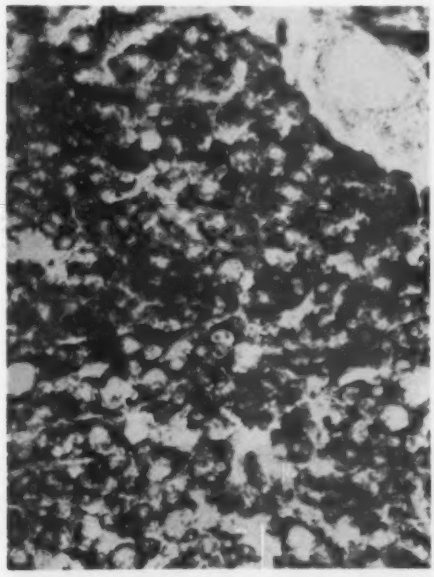


Fig. 9.—Diffusely fatty liver (alcoholic). Spheres of fat are rimmed by stained cytoplasm. There is a portal tract at upper right. SHD; × 125.

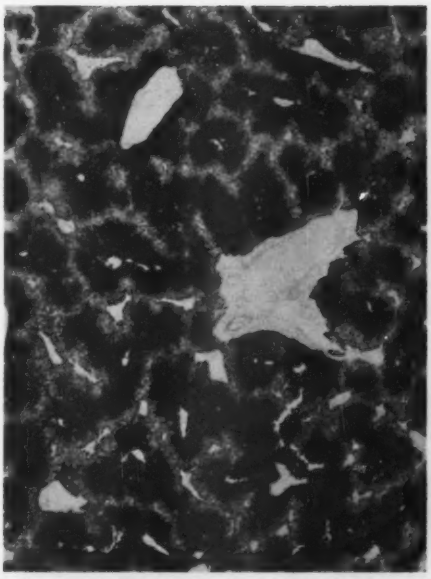
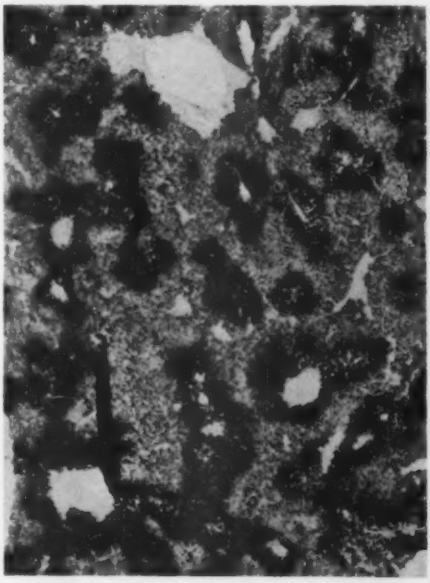


Fig. 10.—Diffusely fatty liver, postmortem specimen (alcoholic). Enzyme activity is reduced periportally. SHD; × 9.

Fig. 11.—Fatty liver, surgical biopsy specimen (nonalcoholic). Note same pattern of enzyme activity as in Figure 10. The fat was limited to the centers of lobules, where enzyme activity was greatest. SHD; × 7.1.



Alcoholism (Table 2).—Enzyme activity was normal in livers of alcoholic patients in whom the livers either were histologically normal or showed portal fibrosis or inactive

cirrhosis. In one autopsy case (Fig. 12) staining for both enzymes was reduced toward the center of the nodules of liver cells. This patient had died of massive gastrointestinal hemorrhage.

The patterns of enzyme activity in fatty livers without cirrhosis varied. In two fatty livers obtained post mortem staining was uniform throughout the liver parenchyma. One of these had broad zones of postnecrotic scarring. In five cases focal areas of liver cells did not stain for DPNHD activity (Fig. 7). Within these foci of nonstaining liver cells, individual liver cells showed evidence of enzyme activity (Fig. 8). The SHD staining was uniform or reduced periportally in four of these cases (Fig. 9). In the remaining one some foci did not stain for SHD activity. In one there was little evidence of SHD activity peripherally, but staining for DPNHD activity was uniform (Fig. 10).

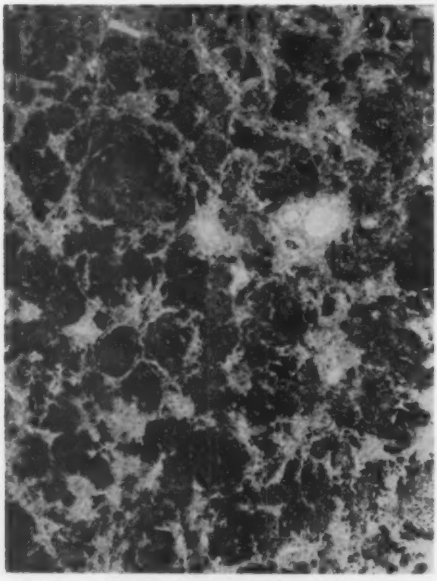
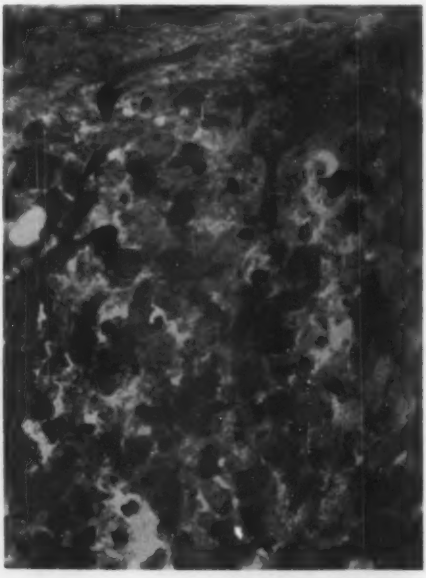


Fig. 12.—Inactive cirrhosis (alcoholic). Staining is reduced in the center of some groups of hepatic cells. There is no activity in the irregular branching areas of fibrosis. SHD; ×6.8.

In active cirrhosis there were foci in which both enzymes were reduced or absent in the liver taken during autopsy (Figs. 13

Fig. 13.—Active cirrhosis (alcoholic). Absence of enzyme activity is seen in liver cells trapped in scar tissue and in some groups of liver cells. SHD; ×14.2.

Fig. 14.—Active cirrhosis (alcoholic). Individual liver cells show absence of enzyme activity. DPNHD; ×71.



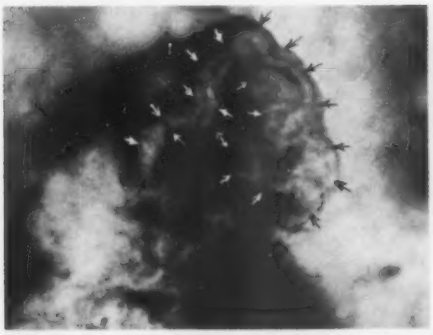


Fig. 15.—Active cirrhosis (alcoholic). The arrows outline curvilinear cytoplasmic masses ("alcoholic hyalin"), which lack enzyme activity. The "alcoholic hyalin" is separated by cytoplasm showing enzyme activity. DPNHD with eosin counterstain; × 1,044.

and 14). This was also observed in many, but not all, specimens removed for biopsy. Staining was often absent or reduced in areas of bile stasis, fatty change, and foci of cells containing "alcoholic hyalin" bodies. When cells containing "alcoholic hyalin" showed enzyme activity, the reduced dye was present within the cell cytoplasm but not within the hyalin masses (Fig. 15).

Summary of Results.—Histologically normal liver cells show uniform SHD and DPNHD activity throughout the liver lobule. Liver cells containing bile plugs, lipofuchsin, and fat in patients without a history of alcoholism showed no reduction in SHD or DPNHD activity. Necrotic liver cells associated with passive congestion or viral hepatitis showed no SHD or DPNHD activity.

Most fatty livers of alcoholic patients contained foci of liver cells which showed no DPNHD activity. These foci differed in two ways from the necrotic liver foci seen in the patients without a history of alcoholism. 1. In the fatty livers of alcoholic patients there was usually focal loss of DPNHD activity without loss of SHD. In contrast, both SHD and DPNHD activity were absent in foci of liver necrosis in the patients without a history of alcoholism. 2. In the fatty livers of alcoholic patients individual liver cells retaining DPNHD activity were found among groups of liver cells showing loss of DPNHD activity. In contrast, DPNHD activity was uniformly

TABLE 2.—*Enzyme Activity in Livers of Patients with a History of Alcoholism*

Case	PM * Hours	NB †	SB ‡	Morphologic Findings	Enzyme Activity DPNHD §	Enzyme Activity SHD ‖
1		x		Normal	Normal	Normal
2,3 ¶		x		Portal fibrosis	Normal	Normal
4		x		Inactive cirrhosis	Normal	Normal
5			x	Inactive cirrhosis	Normal	Normal
6	24			Inactive cirrhosis	Reduced focally	Reduced focally
7			x	Inactive cirrhosis	Reduced centrally	Reduced centrally
8	12			Diffuse fat	Normal	Reduced periportally
9	3			Diffuse fat	Absent focally	Reduced periportally
10	26			Diffuse fat	Absent focally	Normal
11	10			Diffuse fat	Absent focally	Normal
12	11			Diffuse fat with portal fibrosis	Absent focally	Reduced periportally
13	16			Diffuse fat with portal fibrosis	Absent focally	Absent focally
14	18			Diffuse fat	Normal	Normal
15	6			Diffuse fat and postnecrotic scar	Normal	Normal
16	8			Active cirrhosis	Absent focally	Absent focally
17	12			Active cirrhosis	Absent focally	Absent focally
18	5			Active cirrhosis	Absent focally	Absent focally
19		x		Active cirrhosis	Absent focally	Normal
20		x		Active cirrhosis	Normal	Absent focally
21 #		x		Active cirrhosis	Normal	Normal
22			x	Active cirrhosis with metastatic carcinoma	Reduced focally	Reduced focally
23,24 ¶		x		Active cirrhosis	Absent focally	Absent focally
25		x		Active cirrhosis	Normal	Normal

* Post mortem. † Needle biopsy. ‡ Surgical wedge biopsy. § Diphosphopyridine nucleotide dehydrogenase. ‖ Succino dehydrogenase system. ¶ Cases considered together because of identical findings. # Denied alcoholism, history unreliable.

lost in foci of liver necrosis in the patients without a history of alcoholism.

Active cirrhosis in alcoholic patients was associated with focal liver-cell loss of both SHD and DPNHD activity. These foci were located both next to and at a distance from the portal tracts. Individual liver cells retaining enzyme activity were present within these foci.

Comment

The pathogenesis of metabolic cirrhosis and associated liver damage has been the subject of much investigation. However, the role of hepatocellular dehydrogenase in various stages of cirrhosis has received little attention. Several dehydrogenases have been studied by biochemical assay of liver homogenates. In liver biopsy specimens, from malnourished children before and after therapy,[14] liver malic, glutamic, and lactic dehydrogenase, DPNH-cytochrome C reductase, and cytochrome oxidase activity remained unchanged following treatment. Succinoxidase activity increased in liver after dietary therapy for malnutrition.[15] A study of human liver biopsy homogenates in cases of active cirrhosis revealed no change in lactic dehydrogenase; reduction of glycerophosphate dehydrogenase and glutamic dehydrogenase, and increase of glyceraldehyde-3-phosphate dehydrogenase, alcohol dehydrogenase, and glucose-6-phosphate dehydrogenase.[16]

This information gained by biochemical assay of liver homogenates is of limited value, because the cell type and cell contents of liver specimens obtained by needle biopsy are quite different from those of normal liver. Expressing the enzyme activity as units per gram of liver protein probably does not solve this problem. The cirrhotic liver has increased bile ducts, connective tissue, fat-laden liver cells, necrotic liver cells, and inflammatory cells. Schmidt et al.[16] attempted to interpret their results by correlating the findings in homogenates with histologic study of the liver biopsy tissue. In acute hepatitis, acute liver-cell damage, rather than inflammatory-cell infiltrate, explained the reduction in enzyme activity, but in the cases of cirrhosis the role of mesenchymal changes was difficult to evaluate.

In the present study different cell types differed in enzyme activity. Evidence of DPNHD activity was found in mesenchymal cells, bile ducts, and blood vessel smooth muscle, as well as in liver cells, whereas succinic dehydrogenase activity was largely limited to the liver parenchyma. The reduced activity of liver cells showing "alcoholic hyalin" degeneration or fatty change and the comparatively slight activity in scar and inflammatory tissue suggest that these factors could obscure the interpretation of enzyme assays of tissue homogenates. On the other hand, the histochemical method used in the present study is only roughly quantitative. More significant distinctions could perhaps be made if both histochemical and biochemical methods were applied to the same tissue.

Some conclusions may be made from the present study. The histologically normal human liver usually has shown diffuse uniform staining for DPNHD and SHD activity. This differs from the findings in livers of the pig, horse, beef,[3] mouse,[8] and others,[17] in that SHD in many mammals is more active peripherally and DPNHD is more active centrally. In this study a variety of conditions were associated with centrilobular reduced SHD activity, but in no case was DPNHD activity increased centrally. Centrilobular reduction of SHD in the human liver may be nonspecific.

Two patterns of enzyme activity emerged in this study which may help to differentiate pathologic processes in the liver: (1) diffuse reduced staining, centrilobular or periportal, and (2) focal reduced or absent staining. Changes in enzyme activity in necrotic lesions associated with passive congestion and viral hepatitis exemplify the second pattern. In central necrosis discrete pericentral zones of liver-cell inactivity for DPNHD and SHD were found without a zone of transition. None of the liver cells were spared

in these zones, but Kupffer's cells retained DPNHD activity. The changes in SHD are similar to those observed in induced "congestive cirrhosis" in the rabbit.[4]

The focal changes in alcoholic fatty livers and active cirrhosis differed from the discrete areas of inactivity in centrally necrotic livers of nonalcoholic patients. In this study DPNHD activity was absent focally in groups of liver cells in diffusely fatty and active cirrhotic livers of alcoholics. In these areas scattered individual cells showed enzyme activity. SHD activity of these fatty livers often was unchanged. In the cases of active cirrhosis, however, SHD activity was often focally absent, too. These foci of enzyme inactivity might represent Zone 3 of the hepatic acinar unit,[18] thought to be less resistant to injury and not necessarily located about the central vein. This evidence suggests that the mechanism of focal enzyme inactivity in livers of alcoholic patients differs from that in central necrosis associated with passive congestion.

The fact that SHD activity was usually not affected in these fatty livers offers a clue to the cause of the reduction of DPNHD activity. In histochemical studies of kidneys of rats subjected to vascular occlusion and meralluride (Mercuhydrin) poisoning the DPNHD and SHD activities were reduced by ischemia in the same interval of time, and SHD was diminished much earlier in meralluride poisoning.[2] On the other hand, centrilobular hepatic-cell SHD activity was not diminished till 30 hours after carbon tetrachloride poisoning, despite the occurrence of central necrosis within 24 hours.[19] SHD activity was diminished in individual cells, rather than throughout the centrilobular cells. In mouse virus hepatitis, liver succinic dehydrogenase activity decreased in 48 hours concomitant with the onset of cell necrosis.[20] According to histochemical study of infarcted human hearts, SHD activity was diminished as early as two hours after the onset of acute symptoms.[21] In some cases enzyme activity was diminished in myocardial cells which did not show histological evidence of necrosis. The contradictions in these observations may be due to species and organ differences in enzyme activity.

It is of interest that most of the fat-laden liver cells of fatty livers showed no diminution of activity of either enzyme in the cytoplasm surrounding the fat globule. This suggests that the reduction in enzyme activity followed the fatty change and therefore probably did not contribute to the fatty degeneration.

As the cirrhosis progresses, and the fatty liver acquires more intralobular connective tissue and "alcoholic hyalin" degeneration appears, both enzyme activities are progressively reduced. However, the scar formation by itself probably does not account for these changes, since areas of nonstaining liver cells were not encountered in inactive cirrhosis. In active cirrhosis small groups of liver cells incorporated within scar tissue and within regenerative nodules often showed no enzyme activity, especially if there was bile stasis or fatty degeneration. It is noteworthy, however, that in livers showing both foci of "postcollapse" cirrhosis and nodules of fatty degeneration, the enzyme activity of the persisting islands of liver cells in the "postcollapse" zones contained no fat and showed for the most part normal enzyme-activity staining. This observation speaks against the idea that liver fat in florid cirrhosis is diminished by starvation.[22] It implies that local differences to some extent determine enzyme activity and the development of degenerative changes. The work of Popper et al.[23] supports this implication, since anastomosis between portal and hepatic veins could be seen. Their findings suggest that blood bypasses lobular and nodular parenchyma in cirrhosis.

The effect of alcohol ingestion upon the liver dehydrogenase activity is not known; however, there is considerable experimental evidence that dietary imbalance and deficiencies of specific amino acids or proteins lead to the development of fatty liver and the reduction of the activity of certain enzymes.[24,25] In animals force-fed on diets deficient in threonine, the liver lipids increased, but no evidence of a general in-

hibition of protein synthesis in the liver was found.[26] This did not rule out selective inhibition of the synthesis or turnover of one or more liver enzymes in these animals. In animals fed a diet partially deficient in threonine the content of liver DPN was reduced.[27] Improper maintenance of normal DPN metabolism may have been due to the lack of enzyme systems responsible for the reoxidation of DPNH. Liver SHD activity has also been found to be reduced by protein- or lysine-deficient diets.[28]

Since SHD and DPNHD, as well as many other dehydrogenases, are derived from riboflavin,[29] a relative riboflavin deficiency may participate in the development of fatty liver and focal decreased dehydrogenase activity. SHD activity has been found to be reduced in the livers of riboflavin-deficient rats,[5] and DPNHD activity slightly reduced in weanling rats fed a riboflavin-deficient diet for 12 weeks.[6] Of the many enzymes derived from riboflavin and assayed,[30] DPNHD was the most stable, and was therefore regarded as one of the most crucial of the known flavin-derived enzymes. In liver biopsy specimens from children treated for kwashiorkor, xanthine oxidase and D-amino acid oxidase were strikingly increased; there was no significant change in riboflavin, glycolic acid oxidase, DPNH-dehydrogenase, malic dehydrogenase, transaminase, and oxidized pyridine nucleotides. In these cases the red blood cell riboflavin doubled with treatment. In one patient who did not recover on treatment, liver DPNHD activity was low. These observations suggest that the reduction in SHD and DPNHD, and liver-cell degenerative changes, may in part be due to riboflavin deficiency.

Summary

Succinic dehydrogenase system (SHD) and diphosphopyridine nucleotide dehydrogenase activity (DPNHD) in human liver were studied histochemically. Liver biopsy and autopsy specimens were studied to learn the distribution of these enzymes in the human liver in a variety of diseases and in alcoholism. DPNHD activity was focally absent, and SHD activity was usually unchanged in most fatty livers of alcoholics. When intralobular fibrosis, "alcoholic hyalin" degeneration, and fat-laden regenerative nodules were present, both SHD and DPNHD activity were focally absent. The possible roles of malnutrition and ischemia in the pathogenesis of these enzyme changes are briefly discussed.

The author wishes to express his appreciation to Dr. Jackson T. Crane, Dr. Henry D. Moon, and Dr. Harold Harper for the use of their facilities and for their helpful advice. He also gratefully acknowledges the photographic assistance of Mr. Donald Longanecker, and the technical assistance of Mrs. Vivian McClure and Mrs. Darlene Conn.

Department of Pathology, University of California Medical Center (22).

REFERENCES

1. Tominaga, H.: Study on the Experimental Induction of Portal Cirrhosis of the Liver: I. An Experimental Study by Ethyl Alcohol Administration, Tokushima J. Exper. Med. 3:176-190, 1957.

2. Wachstein, M., and Meisel, E.: A Comparative Study of Enzymatic Staining Reactions in the Rat Kidney with Necrobiosis Induced by Ischemia and Nephrotoxic Agents (Mercuhydrin and DL-Serine), J. Histochem. 5:204-220, 1957.

3. Schumacher, H. H.: Histochemical Distribution Pattern of Respiratory Enzymes in the Liver Lobule, Science 125:501-503, 1957.

4. Kawano, H.: Experimental Study on the Histogenesis of "Congestive Cirrhosis" of the Liver with Special Reference to the Histochemical Reactions, Tokushima J. Exper. Med. 3:152-175, 1957.

5. Axelrod, A. E.; Potter, V. R., and Elvehjem, C. A.: Succinoxidase System in Riboflavin-Deficient Rats, J. Biol. Chem. 142:85-87, 1942.

6. Burch, H. B.; Lowry, O. H.; Padilla, A. M., and Combs, A. M.: Effects of Riboflavin Deficiency and Realimentation on Flavin Enzymes of Tissues, J. Biol. Chem. 223:29-45, 1956.

7. Wattenberg, L. W.: A Histochemical Study of 5 Oxidative Enzymes in Carcinoma of the Large Intestine in Man, Am. J. Path. 35:113-137, 1959.

8. Pearson, B.; Grose, F., and Green, R.: Histochemical Changes in Liver Succinic Dehydrogenase During Rapid Growth Following Partial Hepatectomy, Am. J. Path. 35:139-151, 1959.

9. Farber, E., and Louviere, C. D.: Histochemical Localization of Specific Oxidative Enzymes: IV. Soluble Oxidation-Reduction Dyes as Aids in the Histochemical Localization of Oxidative Enzymes with Tetrazolium Salts, J. Histochem. 4:347-356, 1956.

10. Farber, E., and Bueding, E.: Histochemical Localization of Specific Oxidative Enzymes: V. The Dissociation of Succinic Dehydrogenase from Carriers by Lipase and the Specific Histochemical Localization of the Dehydrogenase with Phenazine Methosulfate and Tetrazolium Salts, J. Histochem. 4:357-362, 1956.

11. Nachlas, M. M.; Walker, D. G., and Seligman, A. M.: A Histochemical Method for the Demonstration of Diphosphopyridine Nucleotide Diaphorase, J. Biophys. & Biochem. Cytol. 4:29-38, 1958.

12. Kehrmann, F., and Havas, E.: Zur Kenntnis des Phenazins, Ber. Deutsch. chem. Gesellsch. 46: 341-352, 1913.

13. Protein Malnutrition, proceedings of a conference in Jamaica (1953), sponsored jointly by the Food and Agriculture Organization of the United Nations, the World Health Organization, and the Josiah Macy, Jr. Foundation, edited by J. C. Waterlow, London, Cambridge University Press, 1955, p. 35.

14. Waterlow, J. C., and Patrick, S. J.: Enzyme Activity in Fatty Livers in Human Infants, Ann. New York Acad. Sc. 57:750-763, 1954.

15. Waterlow, J. C.: A Note on the Study of Some Enzymes in Human Liver in Kwashiorkor and in Marasmus, in Liver Function, a Symposium on Approaches to the Quantitative Description of Liver Function, edited by Ralph W. Brauer, Washington, D.C., American Institute of Biological Sciences, 1958, pp. 546-549.

16. Schmidt, E.; Schmidt, F. W., and Wildhirt, E.: Ferment-Aktivitäts-Bestimmung in der Menschlichen Leber: IV. Aktivitäts-Bestimmungen von Enzymen des energieliefernden Stoffwechsels bei chronischen Leber-Entzündungen, Klin. Wchnschr. 36:611-616, 1958.

17. Rutenburg, A. M.; Wolman, M., and Seligman, A. M.: Comparative Distribution of Succinic Dehydrogenase in 6 Mammals and Modification in the Histochemical Technique, J. Histochem. 1:66-81, 1953.

18. Rappaport, A. M.: The Structural and Functional Acinar Unit of the Liver: Some Histopathological Considerations, in Hepatitis Frontiers, edited by F. W. Hartman, G. A. LoGrippo, J. G. Mateer, and J. Barron, Boston, Little, Brown & Company, 1957, pp. 3-17.

19. Leduc, E. H., and Wilson, J. W.: Injury to Liver Cells in Carbon Tetrachloride Poisoning: Histochemical Changes Induced by Carbon Tetrachloride in Mouse Liver Protected by Sulfaguanidine, A.M.A. Arch. Path. 65:147-157, 1958.

20. De Ritis, F.; Coltorti, M., and Giusti, G.: Enzymic Activities of Liver and Serum in Virus Hepatitis of Mice, J. Infect. Dis. 101:219-223, 1957.

21. Wachstein, M., and Meisel, E.: Succinic Dehydrogenase Activity in Myocardial Infarction and in Induced Myocardial Necrosis, Am. J. Path. 31:353-365, 1955.

22. Popper, H., and Szanto, P. B.: Fatty Liver with Hepatic Failure in Alcoholics, J. Mt. Sinai Hosp. New York 24:1121-1131, 1957.

23. Popper, H.; Elias, H., and Petty, D. E.: Vascular Pattern of the Cirrhotic Liver, Am. J. Clin. Path. 22:717-729, 1952.

24. Sidransky, H., and Farber, E.: Chemical Pathology of Acute Amino Acid Deficiencies: I. Morphologic Changes in Rats Fed Threonine-, Methionine-, or Histidine-Devoid Diets, A.M.A. Arch. Path. 66:119-134, 1958.

25. Van Pilsum, J. F.; Speyer, J. F., and Samuels, L. T.: Essential Amino Acid Deficiency and Enzyme Activity, Arch. Biochem. 68:42-53, 1957.

26. Sidransky, H., and Farber, E.: Chemical Pathology of Acute Amino Acid Deficiencies: II. Biochemical Changes in Rats Fed Threonine- or Methionine-Devoid Diets, A.M.A. Arch. Path. 66: 135-149, 1958.

27. Arata, D.; Svenneby, G.; Williams, J. N., Jr., and Elvehjem, C. A.: Metabolic Factors and the Development of Fatty Livers in Partial Threonine Deficiency, J. Biol. Chem. 219:327-333, 1956.

28. Prigmore, J. R.; Bothwell, J. W., and Williams, J. N., Jr.: Response of Liver Enzymes and Other Proteins to Amino Acid Deficient Diets, Proc. Soc. Exper. Biol. & Med. 88:43-46, 1955.

29. Mahler, H. R.: Biological Oxidations, Ann. Rev. Biochem. 26:17-62, 1957.

30. Burch, H. B.; Arroyave, G.; Schwartz, R.; Padilla, A. M.; Béhar, M.; Viteri, F., and Scrimshaw, N. S.: Biochemical Changes in the Liver Associated with Kwashiorkor, J. Clin. Invest. 36: 1579-1587, 1957.

Regeneration of the Fundic Mucosa in Rats

IV. Behavior of the Connective Tissue Under Various Hormonal Influences

EIVIND MYHRE, M.D., Oslo, Norway

The epithelial regeneration of defects produced in the glandular stomach of rats under various hormonal influences has been described in three previous papers.[1-3] In the two first articles it was concluded that no difference could be demonstrated in the epithelial healing capacity of the defects. This conclusion applied to all groups of animals, comprising the following hormonal imbalances: administration of estrogen, androgen, thyroxin, adrenocorticotropin, cortisone, and growth hormone, as well as castration, hypophysectomy, adrenalectomy, exposure to stress, and thyroid deficiency produced by means of radioiodine. The third article, however, revealed that sufficiently large doses of corticotropin and cortisone were capable of causing retarded epithelial covering of the defects.

The purpose of the present study is to investigate the behavior of the connective tissue of the defects in the same groups of animals. In recent years the connective tissue has attracted increasing attention, and much more interest has been directed toward hormonal control of its function and reactivity.[4] It is difficult to recognize specific hormonal effects in single organs or special tissues, since other endocrine imbalances may result from the application of a single hormone.

Although wound healing may be regarded primarily as the formation of new connective tissue[5] and new vessels, it also includes epithelization. The differentiated connective tissues of the body cannot dedifferentiate to give origin to the young cells that start fibroplasia. Instead, a group of mobile cells come from other sources of supply. Fibroplasia starts a few days after wounding, with the appearance and multiplication of undifferentiated mesenchymal cells and fibroblasts. These cells differentiate until they reach the mature state, which they ultimately maintain. The connective tissue also includes the following cells: histiocytes, mast cells, fat cells, lymphocytes, plasma cells, and occasionally neutrophils. The nature, genesis, and interrelation of the connective tissue fibers—collagen, reticulin, elastin, and procollagen—are still obscure. Extensive investigations have been attached to these problems.[6-8] Many studies have also been devoted to the genesis and function of the protein-rich amorphous ground substance. Several components are known, and thus many mucopolysaccharides.[9] The information obtained from the comprehensive literature regarding the connective tissue, its morphology and function, histochemistry, and hormonal influence, will not be repeated here. Reference is made to the works of Asboe-Hansen,[5] Tunbridge,[10] Klemperer,[11] and Florey.[12]

Materials and Methods

The material consists of the animal groups previously described in connection with epithelial healing.[1-3] It comprises a total of more than 600 rats, with equal numbers of males and females. The technique employed in the gastric operation and the preparation of specimens, as well as other procedures, operations, and injections, are fully described in my previous papers. It will be recalled, however, that the gastric defect was produced by excision one week after the various hormonal imbalances had been induced. The ma-

Submitted for publication June 9, 1959.

From Institutt for Patologisk Anatomi, Rikshospitalet; Chief: Prof. Olav Torgersen, M.D.

terial consists, besides the control groups, of animals subjected to the following measures: administration of estrogen, androgen, growth hormone, corticotropin, and cortisone, in various doses, thyroxin, and radioiodine, as well as castration, hypophysectomy, adrenalectomy, and exposure to stress.

The 15th day after the gastric operation has been found to be the most suitable time for comparative studies of epithelial healing. Particular emphasis will be laid, therefore, on observations regarding the connective tissue made at this time. Each of the groups observed on the 15th postoperative day comprise eight rats. However, each of the groups referred to in the latest paper, viz., the animals treated with larger doses of corticotropin and cortisone and those exposed to stress, comprise 16 rats.

In addition to the routine staining methods, viz., hematoxylin-eosin and mucicarmine, the following staining methods have been employed for special investigation of the connective tissue components: Mallory's collagen stain, Gomori's silver impregnation method for reticulin, Weigert's resorcin fuchsin, the periodic acid-Schiff method for mucopolysaccharides, phosphotungstic acid hematoxylin for fibrin, and pinacyanol erythrosinate[18] for mast cells.

In order to avoid predetermined observations during microscopy, corresponding fields of granulation tissue at the base of the defects were selected in the slides by means of the lowest magnifying lens. The area thus examined (Fig. 1) seemed particularly well suited for comparison, being situated between the two folds of the muscular coat produced by the suturation of the defect. The observations were then made with higher magnifications, × 80 and × 320, in the same fields. Three sections taken from the area of the defect were embedded in paraffin and sectioned. Thus, 24 differently stained sections of each defect were available for microscopic study. As the difference was only slight in the various sections from the same animal, it was not difficult to select the representative fields for examination.

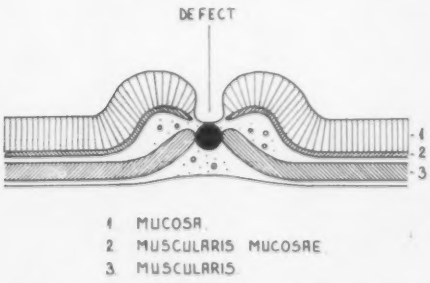


Fig. 1.—Schematic drawing of the defect in the fundic wall. The black circle shows the granulation tissue examined.

The following components were recorded: vessels; fibroblasts; macrophages, neutrophilic and eosinophilic leukocytes, lymphocytes, plasma cells, and mast cells; ground substance, collagen, reticulin, and elastin fibers, as well as the granulation tissue as a whole. The estimated amount of most components was recorded using the following scale: +, ++, +++, ++++. Values between + and ++ were regarded as a decrease; values between ++ and +++ as normal, and a value of ++++ as an increase. The fractioned numbers are due to calculation of mean values in the various groups. Mast cells were counted in the granulation tissue and in the mucosa by recording the number at a magnification of 320 times, representing a field of about 0.13 sq. mm. A number of mast cells less than 5 per field was regarded as decreased; between 5 and 15 as within normal limits, and over 15 as increased.

The observations demonstrated only small variations from animal to animal in the same group. A few animals had to be discarded, owing to the presence of small abscesses in the granulation tissue. All registrations concerning the ground substance had to be regarded with reservation, as the aqueous formalin during fixation may have washed away the soluble matrix to some extent. The ground substance is therefore not recorded in the chart (Fig. 2).

Results

Most authors studying connective tissue changes have based their findings upon estimation. This is also the case in the present study, except for the mast cells. However, special attention has been paid to the evaluation of quantity. The main observations are recorded in Figure 2.

The regenerating granulation tissue had the same appearance in the control groups as in the groups treated by estrogen and androgen, as well as in the castrated and adrenalectomized groups. All lesions in these groups presented the usual picture of granulation tissue, as judged by the quantity of connective tissue, and the number of cells, fibers, and vessels.

The following groups revealed connective tissue changes:

The *thyroxin*-treated group showed more abundant granulation tissue than the control groups (Figs. 3 and 4). This applied to the

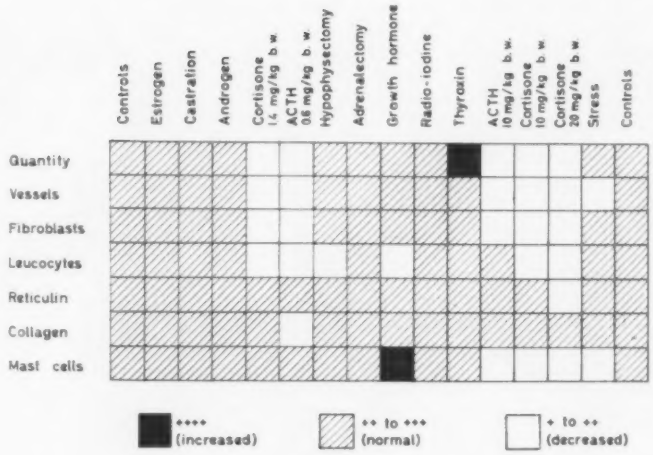


Fig. 2.—The chart shows the quantity of connective tissue as a whole, and of its various components, graded +, ++, +++, ++++. The amount recorded as + is regarded as decreased, that recorded between ++ and +++, as normal, and that recorded as ++++, as increased.

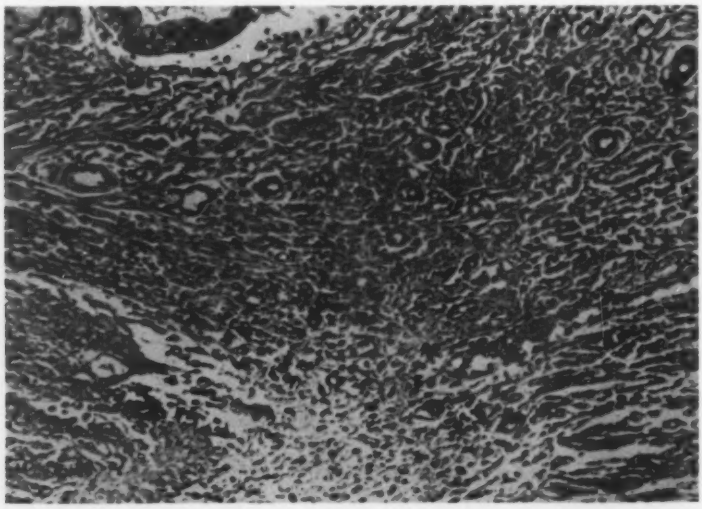
small new vessels, the collagen and reticulin fibers, and the leukocytes.

The *radioiodine*-treated group disclosed a loose, edematous granulation tissue, though no convincing difference in other respects.

The *hypophysectomized* group displayed few lymphocytes and very few granulocytes. No other marked changes were recorded in this group with respect to the components of the granulation tissue, as compared with the controls.

The *corticotropin*-treated animals, with the lower and the higher dosages, all revealed reduced development of connective tissue (Fig. 5). This applied to fibroblasts and vessels, as well as to leukocytes. Further, the amount of collagen fibers was probably diminished. The number of mast cells in the granulation tissue was reduced in the highest-dosage corticotropin-treated animals (Fig. 8), but not in the mucosa.

Fig. 3.—Thyroxin-treated animal with abundant granulation tissue, compared with the control animal (Fig. 4) and with the corticotropin-treated (Fig. 5) and cortisone-treated animals (Fig. 6). Hematoxylin-eosin stain; × 175.



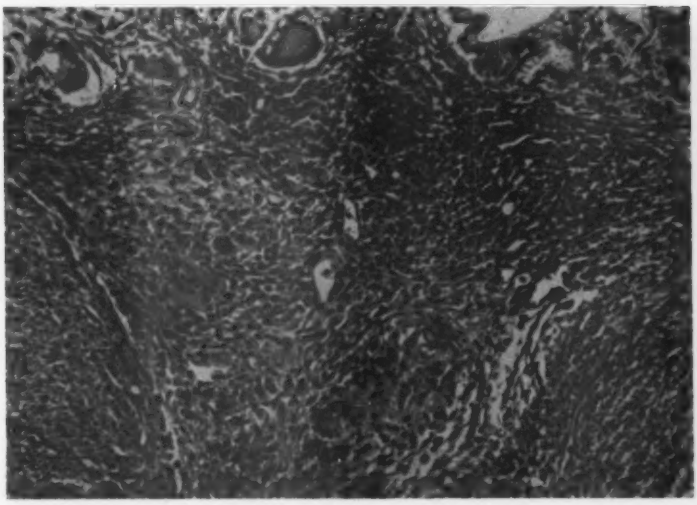


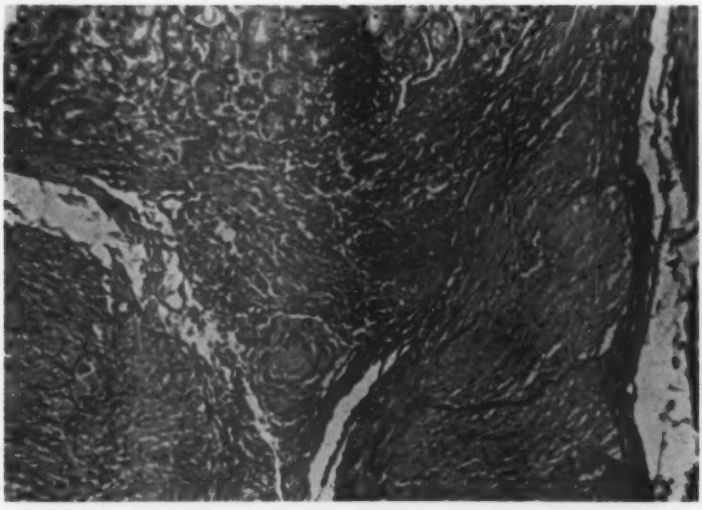
Fig. 4.—Usual amount of granulation tissue in a control animal on the 15th postoperative day. Hematoxylin-eosin stain; × 175.

All *cortisone*-treated groups, like the corticotropin-treated groups, showed a distinctly decreased amount of fibroblasts, vessels, leukocytes, and possibly reticulin fibers (Fig. 6). The two highest-dosage groups revealed a very low mast-cell count, less than 5 cells per field, in the granulation tissue and in the mucosa.

The *stress* group showed reduced development of new vessels and few mast cells, but no definite changes with regard to the other components.

The *growth-hormone*-treated group contained the highest number of mast cells in both locations (Fig. 7). Few leukocytes were noted, but no other changes were observed in the connective tissue.

Fig. 5.—Corticotropin-treated animal, with greatly reduced amount of granulation tissue. Hematoxylin-eosin stain; × 175.



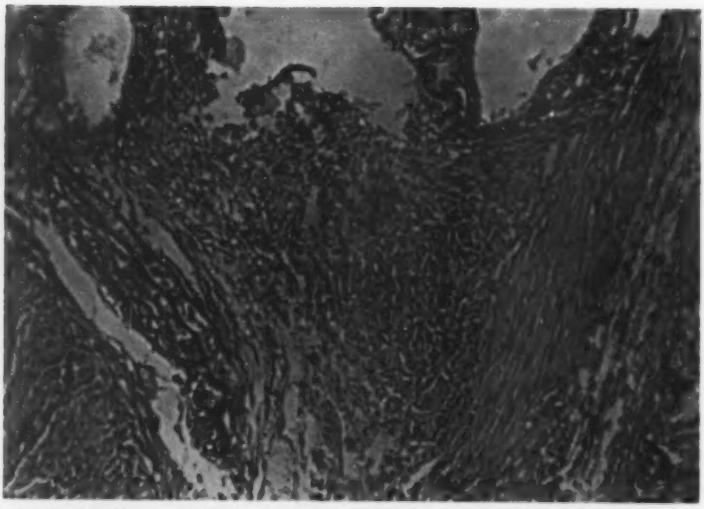


Fig. 6.—Highest-dosage cortisone-treated animal, with very little granulation tissue. Hematoxylin-eosin stain; × 175.

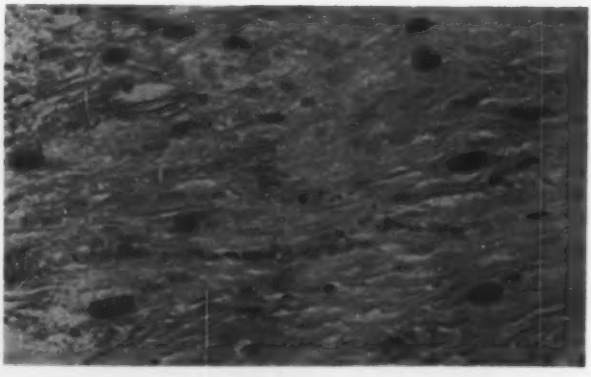


Fig. 7.—Large, well-granulated mast cells in the growth-hormone-treated group. Compare with Figure 8. Pinacyanol-erythrosinate stain; × 450.

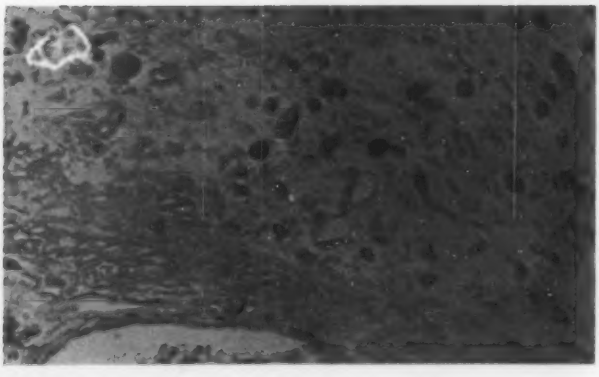


Fig. 8.—Small mast cells with clumped granules in a cortisone-treated animal. Pinacyanol-erythrosinate stain; × 450.

Comment

On the 15th postoperative day the control groups presented the usual picture of regenerating connective tissue. The dominating cell was the fibroblast, but several lymphocytes and some granulocytes and mast cells were also observed. The reticulin fibers always outnumbered the collagen fibers. Elastin fibers were not seen on the 15th day after operation. Many small vessels were present.

Some of the experimental groups seemed to be unaffected by the various hormonal influences, with regard to the formation of granulation tissue at the base of the defect. This is true in the estrogen-treated and androgen-treated groups, and in the castrated and adrenalectomized groups. These hormonal imbalances have been reported in the literature to produce various connective tissue changes. Thus, sex hormones, estradiol and testosterone, have been reported to inhibit granulation tissue surrounding turpentine abscesses in rats.[14,15] Likewise, the difference in collagen content of tissue surrounding tumors in male and female rats has been considered as a possible influence of sex hormones.[16] Inhibition of fibril formation has also been reported following administration of estrogens.[17] Furthermore, decrease of collagen synthesis has been described in female rats as compared with male rats.[18] Fibroblastic response and amount of collagen slightly less than in the control groups have been reported in the granulation tissue of turpentine abscesses in rats after castration and adrenalectomy.[15] The discrepancy between the reported changes of the connective tissue under the hormonal influences mentioned and the lack of changes in this study may be due to several factors, viz., the technique employed, the animal species used, the dosage, and the site of examination. In this respect I should like to emphasize the difficulty in estimating the changes in the connective tissue. It is evident from the literature in this field that there is a lack of exact criteria for accurate quantitative measurements. Slight differences may have passed unnoticed in the present study. On the other hand, it was considered essential that only definite changes should be registered.

The apparent increase of granulation tissue in the thyroxin-treated group seems to correspond poorly with the findings of Moltke,[19] who found inhibited collagen-fiber formation and reduced tensile strength of skin wounds in guinea pigs after thyroxin administration. On the other hand, the reported promoting effect on connective tissue formation of thyroxin treatment[14] is in accordance with the findings in this study.

The absence of a convincing effect of "thyroidectomy" by I^{131}, apart from the edema, corroborates the results of Moltke,[19] who found no difference in tensile strength of skin wounds in guinea pigs between the thyroidectomized group and the controls. It has been suggested that the changes in myxedema may be due not to deficiency of thyroxin but to an excess of thyrotropic hormone.[12]

The reported failure of development of granulation tissue surrounding turpentine abscesses after hypophysectomy[14] has not been verified in this study.

The corticotropin-treated and cortisone-treated animals revealed the most pronounced effect on the connective tissue of all groups. They displayed decreased development of all components of the granulation tissue. This is in accordance with the generally accepted view of the inhibiting effect of these hormones on fibroplasia. Such delay has been demonstrated in healing skin wounds of rabbits and mice after administration of adrenocorticotropic hormone and cortisone.[20-22] Ashton and Cook[23] drew the conclusion that the effect of cortisone on vascularization in the ear chamber of the rabbit is due more probably to its profound effect on circulation than to a direct inhibition of endothelial growth. With regard to experimentally produced gastric ulcers, Janowitz et al.[24] found retardation of ulcer healing in dogs after corticotropin and cortisone treatment. Skoryna et al.[25] found

prolonged persistence of gastric ulcers in rats treated with cortisone, though no qualitative difference could be detected in the fibrous tissue formation.

Stress induced by various means has been shown to retard the healing of laparotomy wounds in rats as measured by the bursting pressure.[26] In this study the stress group displayed decreased formation of vessels and reduced number of mast cells. The connective tissue changes, however, were not so prominent as in the corticotropin-treated group. The stress was severe, as evaluated by the general effect on the animals. Thus, these rats did not gain weight and were in a bad general condition during the experiments. However, the adrenals increased in weight only by 20% during the period from gastric operation to autopsy, while the corresponding increase in the corticotropin-treated animals was 35%. This difference was not found to be statistically significant, however.[3]

Growth-hormone administration has been reported to stimulate fibroblastic proliferation in rats.[14] This observation was not confirmed in this study. The only definite difference in connective tissue from the control groups was the increase in number of mast cells. This is in agreement with other experiments published.[10] Some of these were carried out, however, with anterior pituitary extract containing some thyrotropin.[4]

Mast cells seem to be very active, as they are thought to be associated with sulfate metabolism, heparin, and/or hyaluronic acid and histamine release. During wound healing the mast-cell count is generally increased considerably in the immediate neighborhood of the wound.[27] The ground substance is PAS-positive, and so are many of the granules of the mast cells. Thus, it seems possible that the mast cells are responsible for the production of the ground substance, or at least a part of it. Asboe-Hansen has put forward much evidence to suggest that the mast cell is the source of the connective tissue mucopolysaccharides.[5] The ground substance may, in turn, be a conditioning factor in the formation of other components of the connective tissue. This hypothesis is interesting in connection with the findings in the corticotropin-treated and cortisone-treated groups reported in the present study. The decreased mast-cell count in the highest-dosage groups is in agreement with the findings of Asboe-Hansen[5] and others, though some authors have not been able to confirm these findings (for details see Asboe-Hansen[4]). If these compounds act primarily upon the mast cells, it would be possible, in view of the above-mentioned theory, to explain the lack of development of granulation tissue in animals treated with corticotropin and cortisone. A suggestion in the same direction is the high number of large, well-granulated mast cells in the growth-hormone-treated group. Further, there may be qualitative changes as well. This is suggested by the observation of faintly stained granules and clusters of granules in small mast cells in the cortisone-treated groups. Other authors[4,10] have also reported qualitative changes in mast cells during treatment with these hormones.

Comparison of Epithelization and Connective Tissue Changes.—It is a common opinion that epithelization of wounds is dependent on the granulation tissue, and that the granulations must be of just the right height to allow the epithelial cells to spread across the surface.[5] The present investigation indicates that epithelial healing of a gastric lesion may occur despite defective formation of granulation tissue (Fig. 9). Corresponding observations have been made by Cramer and Nadel[28] in the mouse stomach and by Spain et al.[22] in the mouse skin. Moreover, the present study seems to indicate that, while decreased formation of granulation tissue was observed in all groups treated with corticotropin and cortisone, only the highest-dosage groups revealed retarded epithelial healing.

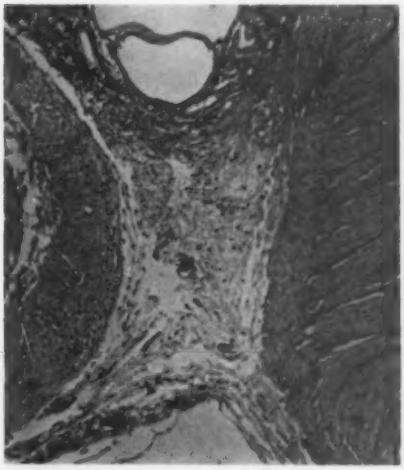


Fig. 9.—Epithelial healing of the defect, in spite of greatly reduced granulation tissue, in a cortisone-treated animal. Hematoxylin-eosin stain; × 70.

Summary

The healing of the connective tissue at the base of experimentally produced defects in the glandular stomach has been studied in rats subjected to various hormonal influences.

Estrogen and androgen administration, castration, and adrenalectomy did not produce demonstrable quantitative or qualitative connective tissue changes.

Minor quantitative changes of the connective tissue were found in some groups of animals. Abundance of all connective tissue components was observed in the thyroxin-treated group. The animals with I^{131}-induced thyroid deficiency disclosed edematous granulation tissue but no other convincing changes. Few leukocytes were observed in the hypophysectomized group and in the growth-hormone-treated group. The stress group showed reduced number of vessels and few mast cells. The changes in the stress group were smaller than in the corticotropin-treated group.

The most marked changes were found in the corticotropin-treated and the cortisone-treated groups, all of which displayed severely inhibited formation of granulation tissue. This applied to the entire amount of connective tissue, as well as to the fibroblasts, leukocytes, vessels, and probably the collagen and reticulin fibers.

The mast-cell count was increased in the growth-hormone-treated animals and highly decreased in the highest-dosage corticotropin-treated and cortisone-treated animals. The possible role of the mast cell in the formation of the ground substance is discussed.

A few animals demonstrated that epithelization may occur in spite of highly decreased formation of granulation tissue in high-dosage cortisone-treated animals.

Institutt for Patologisk Anatomi, Universitet Rikshospitalet.

REFERENCES

1. Myhre, E.: Regeneration of the Fundic Mucosa in Rats: Effect of Estrone and of Castration, A.M.A. Arch. Path. 62:30, 1956.
2. Myhre, E.: Regeneration of the Fundic Mucosa in Rats: II. Effect of Various Hormones and of Ablation of Endocrine Glands on Epithelization, A.M.A. Arch. Path. 67:47, 1959.
3. Myhre, E.: Regeneration of the Fundic Mucosa in Rats: III. Further Studies of the Effect of Corticotropin and Cortisone and of Stress, A.M.A. Arch. Path. 68:134, 1959.
4. Asboe-Hansen, G.: Hormonal Effects on Connective Tissue, Physiol. Rev. 38:446, 1958.
5. Asboe-Hansen, G.: Connective Tissue in Health and Disease, Copenhagen, Ejnar Munksgaard Forlag, 1954.
6. Hall, D. A.; Keech, M. K.; Reed, R.; Saxl, H.; Tunbridge, R. E., and Wood, M. J.: Collagen and Elastin in Connective Tissue, J. Gerontol. 10:388, 1955.
7. Ragan, C., Editor: Connective Tissues, Transactions of the First Conference, New York, Josiah Macy, Jr. Foundation, 1951.
8. Tunbridge, R. E.; Tattersall, R. N.; Hall, D. A.; Astbury, W. T., and Reed, R.: The Fibrous Structure of Normal and Abnormal Human Skin, Clin. Sc. 11:315, 1952.
9. Tunbridge, R. E.: The Connective Tissue System, Ann. Rheumat. Dis. 16:6, 1957.
10. Tunbridge, R. E.: Connective Tissue: A Symposium, Oxford, England, Blackwell Scientific Publications, 1957.
11. Klemperer, P.: The Concept of Collagen Diseases, Am. J. Path. 16:505, 1950.
12. Florey, H. W.: Lectures on General Pathology, delivered at the Sir William Dunn School of Pathology, London, Lloyd-Luke (Medical Books), Ltd., 1954.

13. Bensley, S. H.: Pinacyanol Erythrosinate as a Stain for Mast Cells, Stain Tech. 27:269, 1952.

14. Taubenhaus, M., and Amromin, G. D.: The Effects of the Hypophysis, Thyroid, Sex Steroids, and the Adrenal Cortex upon Granulation Tissue, J. Lab. & Clin. Med. 36:7, 1950.

15. Taubenhaus, M., and Amromin, G. D.: Influence of Steroid Hormones on Granulation Tissue, Endocrinology 44:359, 1949.

16. Grabowska, M.: Collagen Content of Normal Connective Tissue, of Tissue Surrounding a Tumour and of Growing Rat Sarcoma, Nature, London 183:1186, 1959.

17. de Brux, J., and du Boistesselin, R.: Action de différentes hormones sur la morphologie des fibres collagènes: Étude préliminaire au microscope à contraste de phase, Arch. anat. micr. 44: 20, 1955.

18. Tang Kao, K. Y.; Boucek, R. J., and Noble, N. J.: Rate of Collagen Formation in Biopsy-Connective Tissue of the Rat, Proc. Soc. Exper. Biol. & Med. 95:535, 1957.

19. Moltke, E.: Experimental Investigations into the Influence of Thyroxine on Healing Wounds, Copenhagen, Ejnar Munksgaard, Forlag, 1958.

20. Ragan, C.; Howes, E. L.; Plotz, C. M.; Meyer, K., and Blunt, J. W.: Effect of Cortisone on Production of Granulation Tissue in the Rabbit, Proc. Soc. Exper. Biol. & Med. 72:718, 1949.

21. Ragan, C.; Howes, E. L.; Plotz, C. M.; Meyer, K.; Blunt, J. W., and Lattes, R.: The Effect of ACTH and Cortisone on Connective Tissue, Bull. New York Acad. Med. 26:251, 1950.

22. Spain, D. M.; Molomut, N., and Haber, A.: Effect of Cortisone on the Formation of Granulation Tissue in Mice, Am. J. Path. 26:710, 1950.

23. Ashton, N., and Cook, C.: In Vivo Observations of the Effects of Cortisone upon the Blood Vessels in Rabbit Ear Chambers, Brit. J. Exper. Path. 33:445, 1952.

24. Janowitz, H. D.; Weinstein, V. A.; Shaer, R. G.; Cereghini, J. F., and Hollander, F.: The Effect of Cortisone and Corticotropin on the Healing of Gastric Ulcer: An Experimental Study, Gastroenterology 34:11, 1958.

25. Skoryna, S. C.; Webster, D. R., and Kahn, D. S.: A New Method of Production of Experimental Gastric Ulcer: The Effects of Hormonal Factors on Healing, Gastroenterology 34:1, 1958.

26. Chassin, J. L.; McDougall, H. A.; MacKay, M., and Localio, S. A.: Effect of Stress upon the Healing of Wounds in Rats, Proc. Soc. Exper. Biol. & Med. 83:798, 1953.

27. Wichmann, B. E.: The Mast Cell Count During the Process of Wound Healing: Experimental Investigation in Rats, Acta path. & microbiol. scandinav. (Supp. 108), p. 1, 1955.

28. Cramer, L. M., and Nadel, E. M.: Effect of Prednisone upon Gastric Wound Healing, Am. J. Path. 32:659, 1956.

The Fine Structure of the Glomerulus in Amyloidosis

HENRY Z. MOVAT, M.D., Ph.D., Toronto, Canada

The site of localization of amyloid in the glomerulus and its mode of development have been debated for many years. Some [3,4,8,12] believe that it is *deposited* on the inner surface of the basement membrane, or, more precisely, between "the epithelial and endothelial basement membranes." [4] Others [1,2,5] are of the opinion that amyloidosis of the glomerulus is an intrinsic *alteration* ("degeneration") of the basement membrane. The latter view has been expressed also in recent electron microscopic studies.[6,7,13]

The findings to be reported deal with the fine structural alteration of the kidney in three patients with amyloidosis.

Material and Methods

The tissues were obtained by needle biopsy. Two cases were of "primary" and one of "secondary" amyloidosis. In the latter, bronchiectasis was the primary disease. One case of "primary" amyloidosis was mild; the other case and the case of "secondary" amyloidosis were advanced. In the advanced case of primary amyloidosis autopsy was performed four months after the biopsy specimen had been taken. In all three cases a small fragment from each end of the biopsy sample was fixed in buffered (phosphate, pH 7.4) 10% formalin and the rest in Palade's buffered (barbital [Veronal] acetate, pH 7.4) osmium tetroxide. The tissues were fixed for 30 minutes at 4 C, followed by fixation for one hour at room temperature. After dehydration and infiltration, they were embedded in a mixture of butyl- and methylmethacrylate (8:1). Sections were cut with a Porter-Blum (Servall) microtome. Some sections were examined without further treatment; others were impregnated with silver. The sections were examined with an RCA-EMU2 electron microscope. For light microscopy, sections fixed in osmium tetroxide were cut at 0.25μ-0.5μ and impregnated with silver methenamine. Formalin and osmium-fixed material, cut at 2μ-3μ, was stained with hematoxylin (or azure) and eosin, methyl violet, and colloidal iron-PAS.

Submitted for publication June 22, 1959.

Supported by a grant (MA-719) from the National Research Council of Canada.

From the Department of Pathology, University of Toronto Faculty of Medicine, Banting Institute.

Assistant Professor of Pathology; Senior Research Fellow of the Canadian Arthritis and Rheumatism Society.

Results

The diagnosis of renal amyloidosis was based on the morphological appearance of the glomeruli and the typical metachromatic staining with methyl violet (Fig. 1). There was diffuse involvement of the glomeruli and of vessels. Both the autopsy and the biopsy material of the same kidney showed marked narrowing of many capillary lumina and complete obliteration in others (Fig. 2). In one case much of the amyloid was deposited in the hilar area of the glomerulus, i.e., in the first branches of the hilar vessels (Fig. 3). Thin sections impregnated with silver methenamine showed an intact basement membrane with amyloid in the lumen of capillaries, but amyloid was also present outside the basement membrane. In the latter location the amyloid was often present in the form of projections arranged in palisades (Fig. 4).

Amyloid.—Electron micrographs extended the findings obtained with thin silver-impregnated sections examined by light microscopy. The changes were basically the same in all three cases and will be described together. In the hilar area the amyloid lay between the basement membrane and the endothelium (Figs. 5 and 6). In the periphery of the glomerulus it was often on both sides of the basement membrane (Fig. 7). In early lesions amyloid lay only on the inner side of the basement membrane, but as the lesion advanced amyloid appeared also outside the basement membrane (Fig. 8). In advanced lesions it was always on

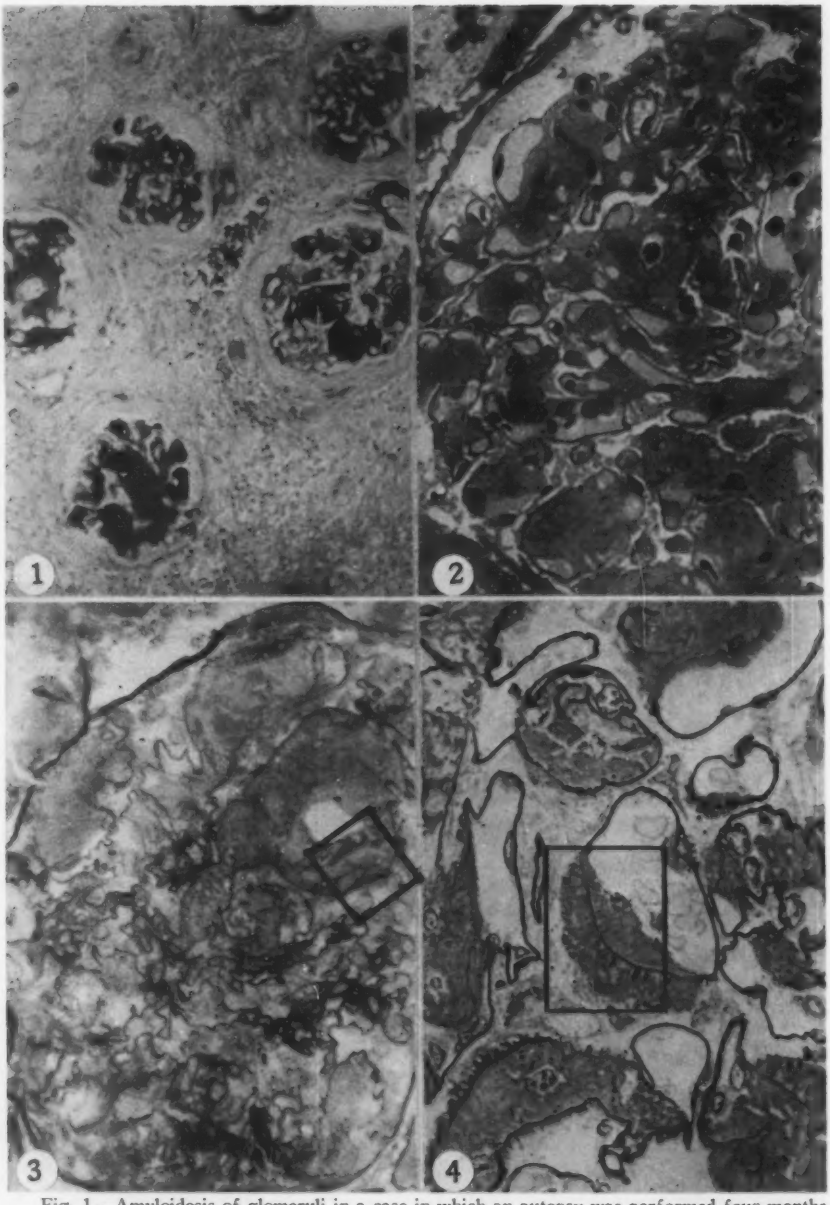


Fig. 1.—Amyloidosis of glomeruli in a case in which an autopsy was performed four months after biopsy specimen had been taken. The amyloid stained purple-red. Formalin fixation, methyl violet; × 83.

Fig. 2.—Glomerulus from case shown in Figure 1, representing material obtained at biopsy. There is diffuse involvement of the glomerulus with narrowing of the capillary lumina. The amyloid appears continuous with the basement membrane (compare with Figure 4). Osmium tetroxide, azure and eosin; × 300.

Fig. 3.—Amyloid deposition mainly in the hilar region of the glomerulus. A branch of a hilar vessel is seen in the right upper portion of the photograph. The outlined area is seen at high magnification in Figure 6. Osmium tetroxide, periodic acid-silver methenamine; × 275.

Fig. 4.—High-power view showing deposition of amyloid on either side of basement membrane. The amyloid outside the basement membrane has a palisade-like appearance. The outlined portion of a capillary loop is seen at higher power in Figure 7. Osmium tetroxide, periodic acid-silver methenamine; × 600.

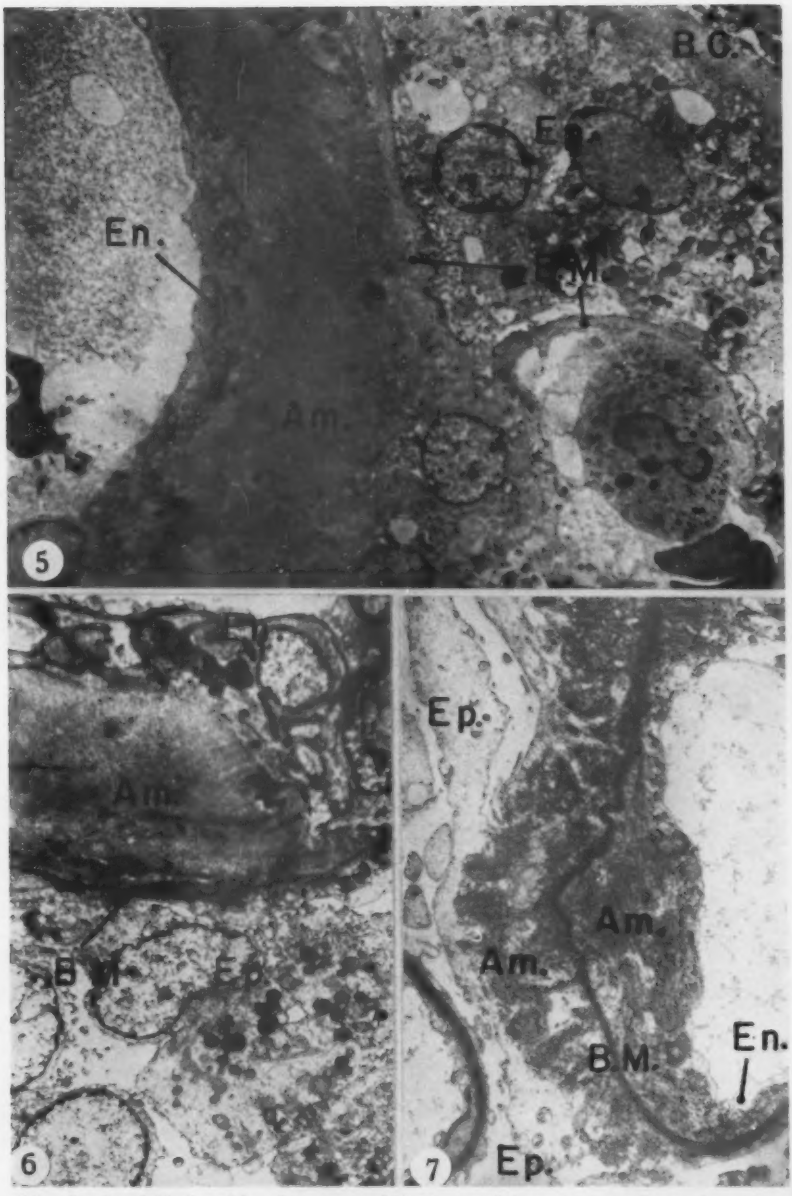
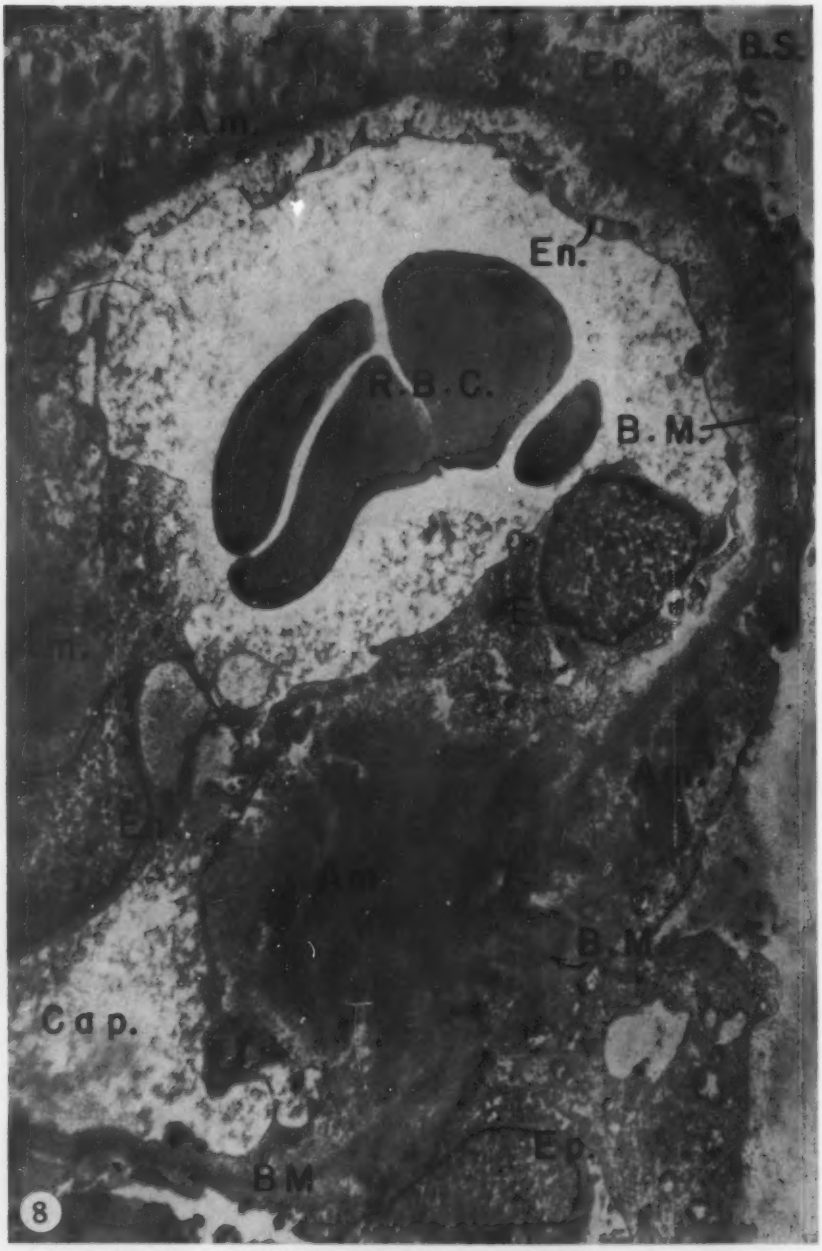


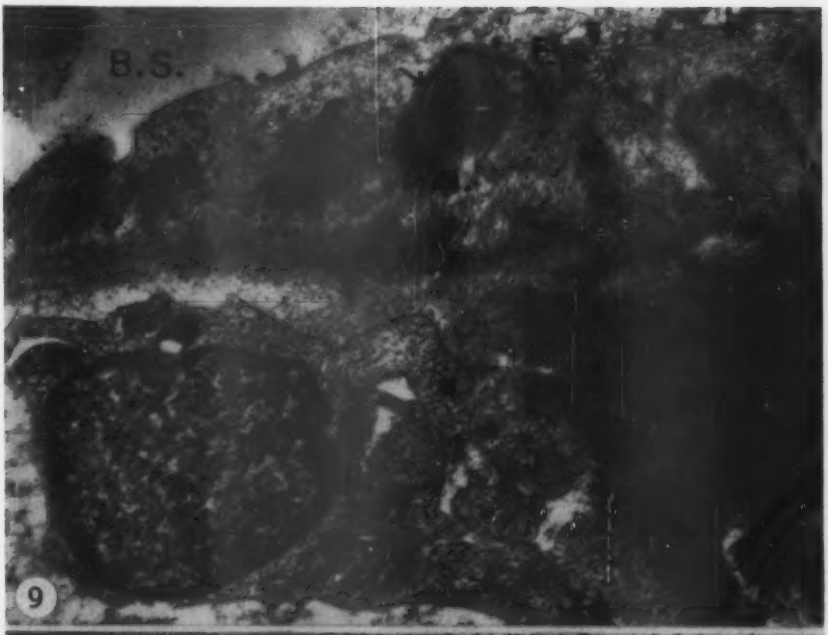
Fig. 5.—Amyloid deposition between endothelium and basement membrane in a branch of the afferent arteriole. The epithelial cells which occupy the right upper quarter of the photograph represent cells covering the glomerulus and those lining Bowman's capsule. They contain hyaline droplets and vacuoles. Osmium tetroxide; × 3,150. *Am.*-amyloid; *B.M.*, basement membrane; *B.C.*, Bowman's capsule; *En.*, endothelium; *Ep.*, epithelium.

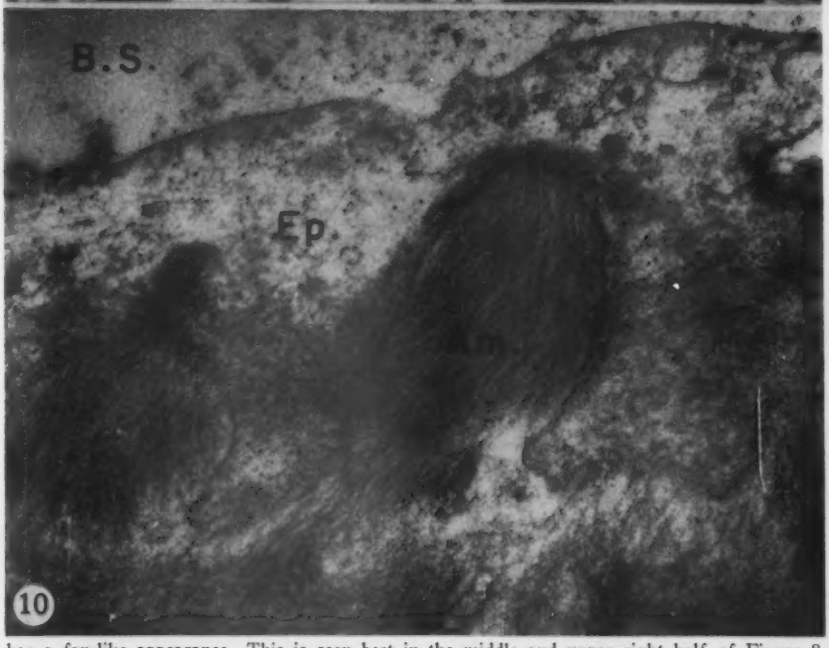
Fig. 6.—Electron micrograph representing a deeper cut through the area outlined in Figure 3. The changes are essentially those described in Figure 5. Osmium tetroxide, periodic acid-silver methenamine, × 2,200. *Am.*-amyloid; *B.M.*, basement membrane; *Ep.*, epithelium; *En.* endothelium.

Fig. 7.—Deeper cut through area outlined in Figure 4. Amyloid is seen on either side of the intact basement membrane, i.e., between endothelium and basement membrane and epithelium and basement membrane. A small portion of a normal capillary loop is present in left lower corner. Osmium tetroxide, periodic acid-silver methenamine; × 2,200. *Am.*-amyloid; *B.M.*, basement membrane; *En.*, endothelium; *Ep.*, epithelium.



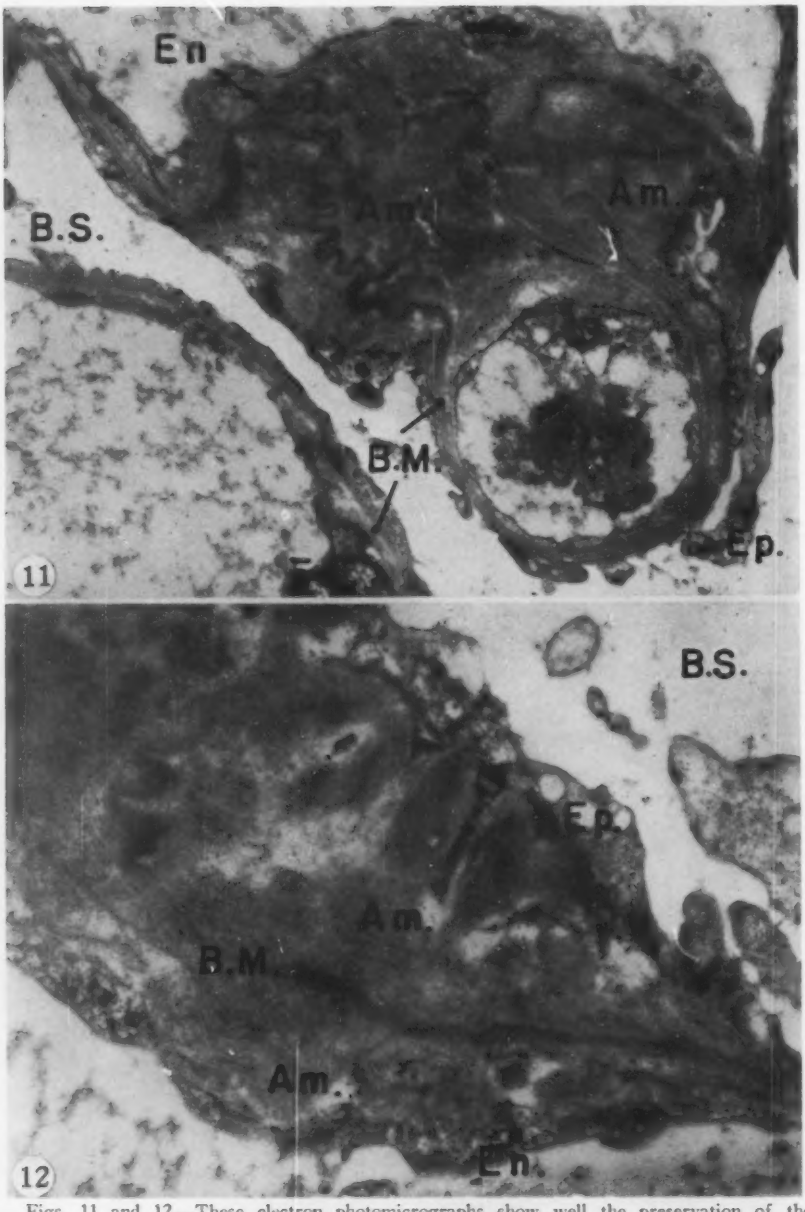
Figs. 8, 9, and 10.—Figure 8 shows a capillary loop and Figures 9 and 10 show an area at higher magnifications. The capillary has an hourglass-like narrowing in the center, due to amyloid deposition between endothelium and basement membrane. There is also amyloid between the basement membrane and the epithelium; the border between the two is seen best in Figure 9 (arrows). Figure 9 also shows details of one endothelial cell and the amyloid between this cell and the basement membrane. The amyloid outside the basement membrane



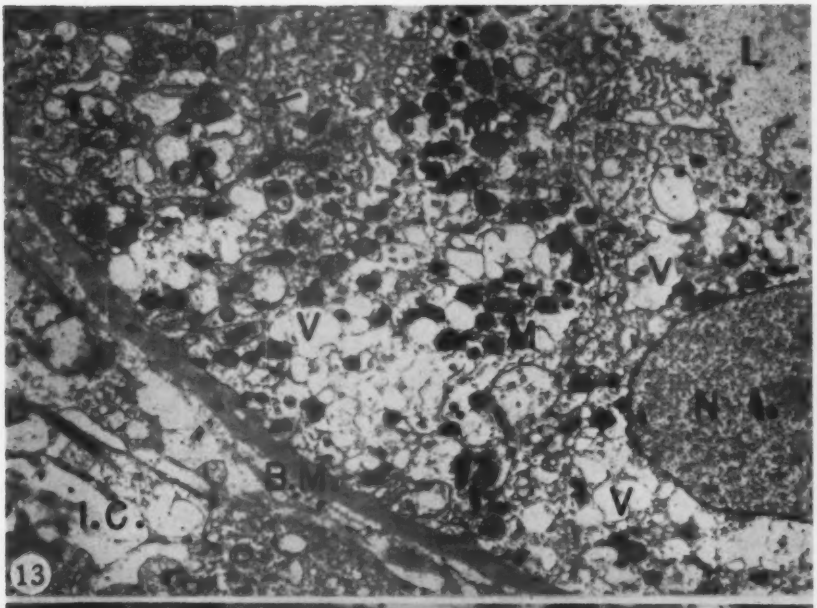


has a fan-like appearance. This is seen best in the middle and upper right half of Figure 8 and is similar to the amyloid seen in Figures 4 and 7. At high magnification (Figures 9 and 10) this amyloid has a filamentous texture. The endothelial cell on the left and the epithelial cell in the right lower corner of Figure 8 contain vacuoles. Osmium tetroxide; strong silver protein U.S.P. (Protargol). Fig. 8, × 6,300; Fig. 9, × 10,800; Fig. 10, × 26,400.

In these figures, *Am.*-amyloid; *B. C.*, basement membrane *B.S.*, Bowman's space; *Cap.*, capillary; *En.*, endothelium; *Ep.*, epithelium; *R.B.C.*, red blood cells.



Figs. 11 and 12.—These electron photomicrographs show well the preservation of the basement membrane, although considerable amount of amyloid is deposited on either side of it. In both photographs there is some waviness of the basement membrane. In the unaffected parts (loop in right lower corner of Figure 11) a lamina densa with laminae rarae on both sides of it can be discerned. Portions of endothelial cytoplasm are present in the mass of amyloid in Figure 11. There are no epithelial foot processes, but an almost continuous sheet of epithelial cytoplasm. In Figure 12 the epithelium adjacent to the amyloid is dense, as the result of compression. Osmium tetroxide, strong silver protein; Fig. 11, ×12,400; Fig. 12, ×13,200. *Am.*-amyloid; *B. M.*, basement membrane; *B.S.*, Bowman's space; *En.*, endothelium; *Ep.*, epithelium.



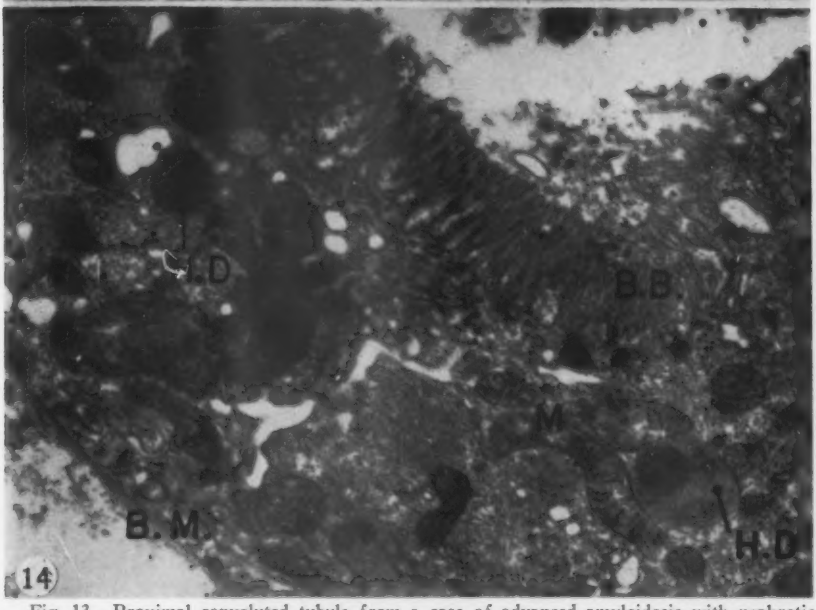


Fig. 13.—Proximal convoluted tubule from a case of advanced amyloidosis with nephrotic syndrome and hypercholesteremia. There are numerous vacuoles in the cytoplasm, many of which are confluent. Some vacuoles have a definite limiting membrane, but others have none. The vacuoles with no membrane probably represent dissolved lipid, whereas those with a membrane contained fluid. The basement membrane is irregular in thickness. The cytoplasmic infoldings at the base of the cell are very scanty. The nucleus and mitochondria are well preserved. The arrows indicate the border between two cells. Osmium tetroxide, strong silver protein; × 4,000. *B.M.*, basement membrane; *I.C.*, interstitial connective tissue; *L*, lumen; *M*, mitochondrion; *N*, nucleus; *V*, vacuole.

Fig. 14.—Proximal convoluted tubule from a milder case of amyloidosis. The brush border is intact in the left three-quarters of the photograph; in the right upper corner (tangenital cut) it is distorted. Two hyaline droplets are present among the mitochondria. The basement membrane is normal. Osmium tetroxide, Protargol; × 19,200. *B.B.*, brush border; *B.M.*, basement membrane; *H.D.*, hyaline droplet; *M*, mitochondrion.

both sides of the basement membrane. The amyloid located inside the basement membrane lay between endothelium and basement membrane (Figs. 8, 9, 11, and 12). At times it had replaced most of the endothelial cell, only fragments of the latter being present in the mass of amyloid (Fig. 11). The amyloid found outside the basement membrane lay between it and the epithelium (Figs. 8-12). The epithelium was pushed away from the basement membrane by the deposited amyloid, at times for a considerable distance (Fig. 12). Some of the amyloid looked homogeneous, but often, particularly small deposits between basement membrane and epithelium, had a filamentous appearance (Figs. 9 and 10).

Basement Membrane.—This structure was found intact in all electron photomicrographs examined. It was particularly well visible in sections impregnated with silver (Fig. 7), but sections impregnated with silver proteinate also showed an intact membrane (Figs. 8, 9, 11, and 12), although slight variations in thickness and undulation were sometimes noted. On the other hand, osmium-fixed, unimpregnated sections showed no clear separation of amyloid from basement membrane.

Epithelium.—In early lesions the foot processes showed disorganization. In advanced lesions no foot processes could be recognized; rather, broad masses of epithelial cytoplasm covered the extravasated amyloid (Figs. 8-12). Compression by the deposit often caused condensation of the epithelium (Fig. 12). Other changes, such as hyaline droplet formation (Fig. 6) and vacuoles (Figs. 5 and 8), were similar to those in membranous glomerulonephritis.[11]

Tubules.—These showed secondary changes, which may possibly be attributed to the hypercholesteremia and proteinuria associated with the nephrotic syndrome. These changes have been described in more detail in another publication.[11] They consisted of accumulation of vacuoles and formation of hyaline droplets. The vacuoles were not confined to any preexisting cytoplasmic structure (Fig. 13). There is little doubt that they represent accumulation of fat, which had been dissolved during dehydration of the tissue with alcohol. The hyaline droplets seem to have developed from mitochondria in which protein had accumulated (Fig. 14).

Comment

The findings in the three cases here reported show, in our opinion quite conclusively, that in amyloidosis the basement membrane of the glomerulus may be very well preserved and that the amyloid does not arise from it. There was no difference between the amyloid of so-called primary and that of secondary amyloidosis. The amyloid was deposited first on the inner surface of the basement membrane, between the latter and the endothelium. As the lesion advanced, amyloid was found also outside the basement membrane, i.e., between the basement membrane and the epithelial cells. The latter were pushed away from the former by the deposits of amyloid. This peculiar deposition on either side of the basement membrane may well explain why some observers believed that amyloid is a swelling or alteration of the basement membrane, rather than a deposition. It may be noted that we observed intact basement membrane with amyloid on either side of it with both the electron microscope and silver-impregnated sections examined with the light microscope. Figure 15 shows diagrammatically the various hypotheses on the localization of amyloid in the glomerulus.

It is our view that amyloid is a hematogenous proteinaceous material containing globulin, and often carbohydrate, deposited in and between elements of connective tissue. This view is supported also by experimental studies and by immunochemical studies on amyloid. Letterer[9] and others have shown that in experimental amyloidosis, hyperglobulinemia and variations in plasma proteins are essential for amyloid formation. The changes in the plasma proteins consist either of formation of new or

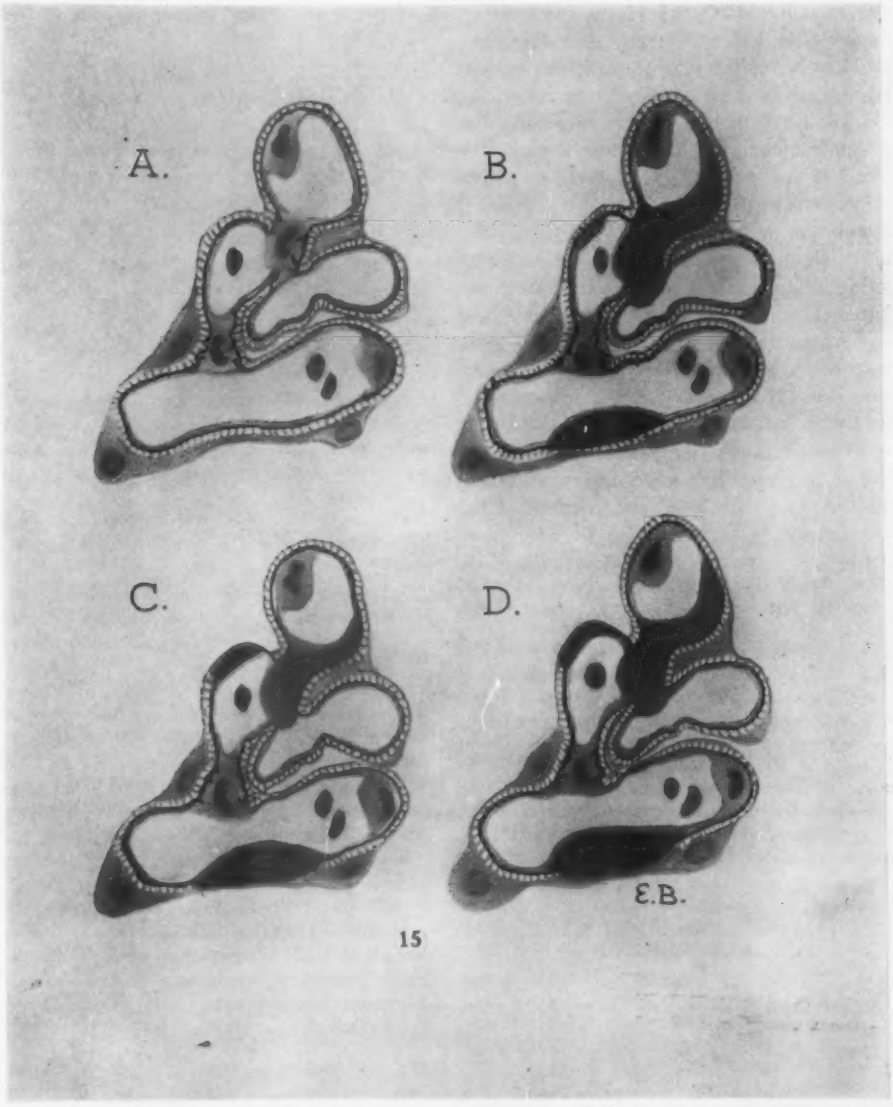


Fig. 15.—Diagram showing the various concepts of amyloidosis of the glomerulus. *A* represents a normal glomerulus; *B* shows amyloid deposited between endothelium and basement membrane; *C*, amyloid as a swelling (degeneration) of the basement membrane, and *D*, amyloid as a deposit between endothelium and basement membrane and between the latter and the epithelium.

abnormal proteins or of a disturbance in their colloidal stability. Mellors and coworkers,[10] using the fluorescent microscope, showed that γ-globulin can be identified in the glomerular deposits of amyloid.

Summary

Tissue obtained by needle biopsy from three cases of amyloidosis was examined by light and by electron microscopy. In the former method, impregnation with silver

methenamine was particularly informative, showing an intact basement membrane with amyloid on either side of it. Thin sections of tissue fixed in osmium tetroxide were floated on either silver methenamine or silver proteinate and examined with the electron microscope. The electron photomicrographs showed that the basement membrane remains intact in amyloidosis, although the amyloid is deposited on either side of it, i.e., between endothelium and basement membrane and between basement membrane and glomerular epithelium. The epithelium showed flattening and fusion of the foot processes in early lesions, and complete destruction in advanced lesions.

These findings indicate that amyloid is not an intrinsic alteration (degeneration and swelling) of the basement membrane, but a deposit.

I wish to express my appreciation to Drs. E. R. Yendt and William Kerr, of the Toronto General Hospital, who provided the renal biopsy tissue, and to Miss Charlotte Turnbull for her patience and skill in the preparation of the many sections examined in this study. Mr. Grant Woodward, of the Department of Physics, University of Toronto, was of invaluable assistance in the operation of the electron microscope. Grateful acknowledgment is made to Mrs. M. Murphy and Miss E. Blackstock, of the Department of Art as Applied to Medicine, for labeling the photographs and for preparation of the diagram, and to Mr. Harold Layne for taking the photomicrographs.

Department of Pathology, University of Toronto, 100 College St. (2).

REFERENCES

1. Allen, A. C.: The Kidney: Medical and Surgical Diseases, New York, Grune & Stratton, Inc., 1951.

2. Allen, A. C.: The Clinicopathologic Meaning of the Nephrotic Syndrome, Am. J. Med. 18:277-314, 1955.

3. Bell, E. T.: Amyloid Disease of the Kidneys, Am. J. Path. 9:185-204, 1933.

4. Churg, J., and Grishman, E.: Phase Microscope Studies of Renal Glomeruli: Glomerular Deposits of "Hyaline" Substances, Am. J. Path. 29:199-215, 1953.

5. Fahr, T.: Pathologische Anatomie des Morbus Brightii, in Handbuch der speziellen pathologischen Anatomie und Histologie, edited by F. Henke and O. Lubarsch, Berlin, Springer Verlag, Band 6, Pt. 1, p. 156-472, 1925.

6. Farquhar, M. G., in Proceedings of the 10th Annual Conference on the Nephrotic Syndrome, to be published.

7. Geer, J. C.; Strong, J. P.; McGill, H. C., Jr., and Muslow, T.: Electron Microscopic Observations on the Localization of Amyloid in the Kidney in Secondary Amyloidosis, Lab. Invest. 7:554-565, 1958.

8. Hueter, C.: Über Amyloid der Glomeruli, Centralbl. allg. Path. u. path. Anat. 19:961-965, 1908.

9. Letterer, E.: Some New Aspects of Experimental Amyloidosis, J. Path. & Bact. 61:496, 1949.

10. Mellors, R. C., and Ortega, L. G.: Analytical Pathology: III. New Observations on the Pathogenesis of Glomerulonephritis, Lipid Nephrosis, Periarteritis Nodosa, and Secondary Amyloidosis in Man, Am. J. Path. 32:455-499, 1956.

11. Movat, H. Z., and McGregor, D. D.: The Fine Structure of the Glomerulus in Membranous Glomerulonephritis (Lipoid Nephrosis) in Adults, Am. J. Clin. Path. July, 1959, in print.

12. Ohmori, D.: Bedeutung der Membrana propria der Glomerulusschlingen in der Nierenpathologie, Arch. path. Anat. 234:53-72, 1921.

13. Spiro, D.: The Structural Basis of Proteinuria in Man: Electron Microscopic Studies of Renal Biopsy Specimens from Patients with Lipoid Nephrosis, Amyloidosis, and Subacute and Chronic Glomerulonephritis, Am. J. Path. 35:47-73, 1959.

Experimental Lathyrism

Effect of Aminoacetonitrile on Connective Tissue Formation in Adult Rats

FRANZ M. ENZINGER, M.D., and E. D. WARNER, M.D., Iowa City

Extensive mesodermal tissue alterations in experimental lathyrism have been reported in recent years. The lesions described have included severe skeletal deformities, spontaneous rupture of the aorta, and hernias.[2-7,12-17] In this work, the growing tissues of immature animals have been especially susceptible. Faulty development of mesodermal tissue in fetuses and a high incidence of fetal death were encountered in pregnant rats maintained on a lathyrogenic diet.[14]

The basic etiology of the lesions has not been established. In the early work lathyrism was induced by feeding a diet rich in the flowering sweet pea (Lathyrus odoratus). Subsequent work has shown that administration of (β-glutamyl-amino) propionitrile (BAN) or aminoacetonitrile (glycinonitrile; AAN) produced similar and severer lesions. The accumulated experimental work has been reviewed by Selye.[13]

Histologically, the more striking lesions showed degeneration of cartilage matrix, proliferation of cartilage cells, slipping of epiphyses, detachment of ligaments, and separation of the periosteum with proliferation of fibroblasts and subsequent formation of exostoses.[2,10-13,17] The occurrence of these lesions in areas of mechanical stress suggested a deficiency in tensile strength of the growing mesodermal tissue. This concept has been supported by experiments in which stress was alleviated by transection of ligaments and muscles,[7] as well as by work on tensile strength of healing wounds,[5] and by studies showing defective collagen fiber formation in healing croton oil pouches [8] and in cartilage.[6] The hydroxyproline content (as index for collagen formation) was found decreased in the croton oil pouches,[8] whereas it was reported unaltered in the lathyritic cartilage.[6]

In the present study, samples of developing connective tissue were obtained by implantation of formalinized polyvinyl sponges.[1] * This method allows growth of new connective tissue under almost no mechanical stress or irritation and lends itself to histologic and chemical study of newly formed connective tissue, which is essentially free of older tissue and which is formed in mature, rather than immature, animals. Aminoacetonitrile (AAN) was used as the lathyrogenic agent.

Methods

The polyvinyl sponges were cut into small cubes of 6-7 mm. diameter and washed in running distilled water for 24 hours to remove the formaldehyde preservative present in the sponge. Immediately before implantation the sponge cubes were sterilized in boiling water for 15 minutes and transferred to sterile isotonic saline.

With the animal under pentobarbital (Nembutal) anesthesia (30 mg/kg. in isotonic saline, administered intraperitoneally), a midline longitudinal incision, approximately 2 cm. long, was made in the abdominal skin of the rat. Three deep, evenly spaced, separate pouches in the subcutaneous space were prepared on each side of the incision by blunt dissection. A cube of polyvinyl sponge was placed in each of the six pouches, and the skin incision was closed.

Sixty-nine mature male albino rats of the Sprague-Dawley strain, weighing 200-400 gm.

Submitted for publication July 2, 1959.

Aided by a grant from the Iowa Division, American Cancer Society.

Department of Pathology, State University of Iowa College of Medicine.

Armed Forces Institute of Pathology, Washington, D.C. (Dr. Enzinger).

* Ivalon Surgical Sponge, Clay Adams Co., New York.

each, were used. All animals received a basic Rockland rat diet † and water ad libitum. The rats were divided into four groups:

Group I (32 rats): Control.

Group II (25 rats): Aminoacetonitrile sulfate (AAN), disolved in isotonic saline and neutralized with sodium bicarbonate, was administered in a daily dose of 50 mg. in 1 ml. by subcutaneous injection.

Group III (6 rats): AAN, 25 mg., given as in Group II.

Group IV (6 rats): Semistarvation. Approximately 5 gm. of Rockland rat diet daily was given to each rat. This group served as a control for evaluation of weight loss encountered in the lathyritic animals.

All animals were weighed on the first and last days of the experiment. Animals were killed for study on the 1st, 3d, 5th, 10th, 14th, 18th, and 25th days after implantation of the polyvinyl sponges. The skin was dissected away from the abdominal wall, and the sponges were carefully freed from the surrounding tissues. One of the six sponges from each animal was placed in 10% formalin solution for histologic studies. The rest of the sponges were placed in acetone and used for chemical studies.

Sponges for histologic study were embedded in paraffin, and routine hematoxylin-eosin stains were made in all instances. In addition, a variety of stains ‡ were made on many of the specimens.

Hydroxyproline and hexosamine determinations were made by the methods of Neuman and Logan[3] and Elson and Morgan,[4] respectively. The sponge-tissue cubes were placed immediately in cold acetone, which was changed after 24 hours. After a minimum of three days in acetone, the sponge cubes were cut into thin slices and freeze-dried. The dried material then was hydrolyzed with hydrochloric acid (3 ml. of 3 N hydrochloric acid added to 100 mg. dry sponge-tissue mixture) in sealed tubes for three hours at 125 C in an autoclave. The acid hydrolysate was neutralized with 3 ml. of 3 N sodium hydroxide, filtered, and diluted to 10 ml. with distilled water. One-milliliter aliquots were used for each hydroxyproline and hexosamine determination. All determinations were made in duplicate. Hydroxy-L-proline § and D-glucosamine ‖ were used for standard solutions. A freshly prepared standard curve and readings obtained on a Klett-Summerson colorimeter were used for calculation of the final values.

Blank control determinations on the polyvinyl sponge alone and on the filter paper yielded negative results for both hydroxyproline and hexosamine. Control hydroxyproline and hexosamine samples were recovered completely after similar autoclaving in hydrochloric acid.

Results

Morphological Observations.—The abdominal skin wounds in both the experimental and the control animals healed well, and major wound infections were not encountered. Although the wounds of lathyritic animals could be pulled apart more easily, spontaneous disruptions of the wounds did not occur.

In sharp contrast to the slight weight gain of controls, the AAN-treated animals lost considerable weight. The weight loss was most pronounced in the first period of AAN administration and in the animals on a regimen of 50 mg. of AAN per day. The weight loss averaged 4-8 gm. daily in animals weighing 250-350 gm. initially. A similar weight loss was achieved in semistarvation controls by restriction of the basic diet.

Although the AAN animals were rather sensitive to touch, they were generally more listless. The fur was ruffled, and in some animals disturbance of gait was evident.

On dissection, the skin of the lathyritic animals appeared thinner and was separated more easily from the abdominal wall. Mild deformations of the vertebral column, sternum, tibia, and femur were observed in the animals that had received AAN 14 or more days. Distention of the intestinal tract by large amounts of semiliquid fecal material was prominent in many animals. Hernias or aneurysms were not encountered. Small hemorrhages were seen at the injection sites occasionally.

† Arcady Farms Milling Company, Chicago.

‡ Van Gieson picrofuchsin (tinnitrophenol and acid fuchsin)
Trichrome (Masson)
Reticulum stains (Snook)
Phosphotungstic acid hematoxylin (PTAH)
Periodic acid-leukofuchsin (Hotchkiss-McManus)
Toluidine blue (1:500 aqueous)
Alcian blue
Colloidal iron (Rinehart and Abul-Haj)
Aldehyde fuchsin (Gomori)

§ California Foundation for Biological Research.

‖ Eastman Chemical Products, Inc., organic chemicals.

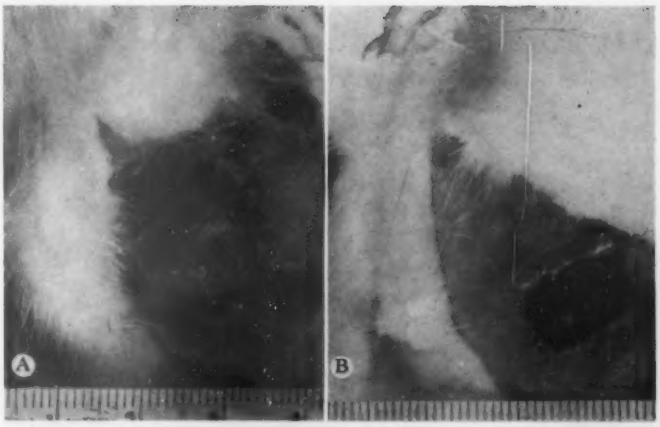


Fig. 1.—Gross appearance of implanted sponge after 10 days. *A*, control; *B*, aminoacetonitrile (AAN), 50 mg. daily. Note well-formed connective tissue capsule of the control.

Differences in the gross appearance of the implanted sponges were most evident in the earlier stages of the experiment. In AAN-treated rats, the sponges were less firmly attached to the abdominal wall and skin, and the poorly formed and rather edematous capsule was unlike the dense and firm capsule of the controls. A reddish-brown discoloration of the implanted sponges was also a prominent feature in most of the AAN-treated animals (Fig. 1). These changes were more prominent in the 50 mg. group than in the 25 mg. group. The gross appearance in the semistarvation controls was indistinguishable from that of controls on unlimited basic diet.

Control Animals: In these animals sections showed active proliferation of fibrovascular tissue about the circumference of the sponge as early as 24 hours after implantation, and in a few instances early fibroblastic invasion of the fibrin-filled spaces of the sponge was present at 24 hours. Capillary buds followed the advancing front of fibroblasts, and by the fifth day loosely arranged fibrovascular tissue filled about two-thirds of the sponge.

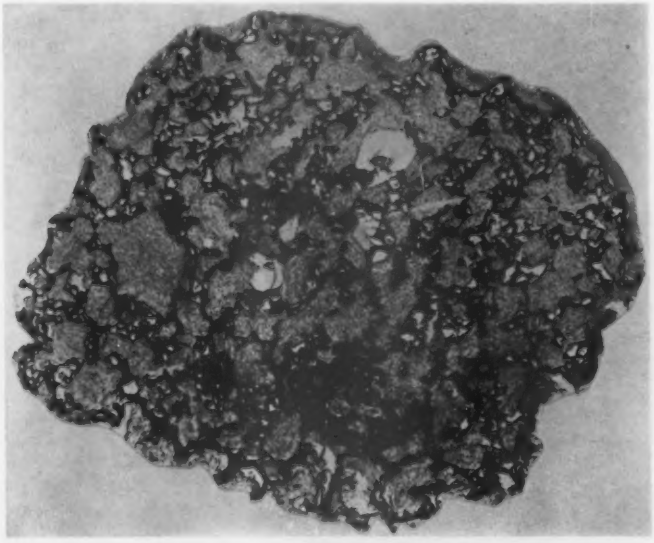


Fig. 2.—New connective-tissue-filled spaces in polyvinyl sponge at 14 days. Control animal. Hematoxylin-eosin stain; reduced to 86% of mag. × 20.

In most instances filling of the sponge was complete by the 14th day (Fig. 2).

Several stages of normal fiber formation could be distinguished. In the earliest stage a homogeneous but irregularly distributed matrix was first laid down between the fibroblasts. The matrix stained well with acidophilic dyes, the periodic acid-Schiff (PAS) reaction, and Alcian blue, and showed some metachromasia with the toluidine blue stain. By the third day, delicate, fibrillary structures became visible within the ground substance, and most of the matrix soon was converted into a dense feltwork of thin, short, and poorly oriented fibers. Although the fibers within the ground substance were formed in the presence of fibroblasts only, no close spatial relationship between fibroblasts and fibril formation was apparent. By the fifth day, the fibers became more prominent, possibly by lateral alignment of the fibrillary elements, but they were still coated with considerable PAS-positive material, which showed no or very little metachromasia. Simultaneously, with increasing length and thickness, reorientation of the fiber elements occurred, and some of the larger fibers were arranged roughly parallel to the sponge septa. With fiber development, the interfibrillary ground substance decreased, and the fibers became separated by narrow and cleft-like spaces.

Rather early, some of the larger fibers showed birefringence and stained deep blue with Masson's trichrome stain and red with Van Gieson's picrofuchsin stain. In reticulum stains, the thin and delicate fibers stained a deep black, while the thicker fibers exhibited a browner tinge. Very few short elastic fibers could be demonstrated with the aldehyde fuchsin stain.

Although capillaries were abundant in the earlier stage, they decreased with progressive fiber differentiation, and vascular elements were relatively scarce 25 days after implantation.

AAN-Treated Animals: The growth of new tissue was retarded during the first 10 days. The capsule about the sponge was comprised of loose, edematous fibrovascular tissue, in contrast to the dense and cellular capsule in the controls. At five days there was relatively little extension of new tissue into the spaces of the sponge. After 10 days the growth into the sponge appeared accelerated, and completely filled sponges were encountered on the 14th day. In both AAN-treated groups, fiber formation did

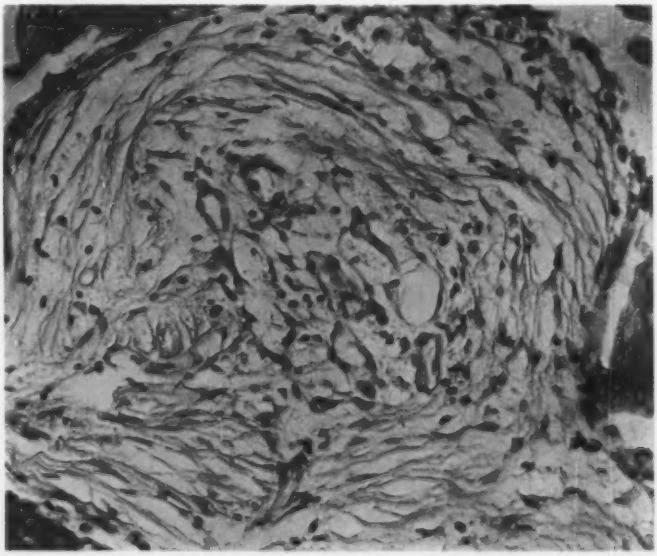


Fig. 3.—Connective tissue at 14 days in control animal. Note well-formed fibers. Hematoxylin-eosin stain; reduced to 85% of mag. × 165

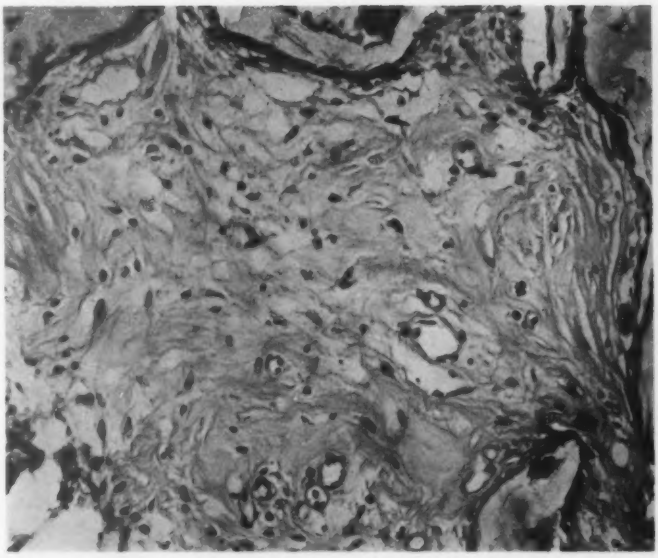


Fig. 4.—Connective tissue at 14 days in AAN-treated animals (25 mg. per day). Fiber formation is minimal, and ground substance remains abundant. Hematoxylin-eosin stain; reduced to 85% of mag. × 165.

not proceed in a normal manner. The fibers remained delicate, and there was little tendency toward aggregation. They were rather short and tended to form a feltwork with little evidence of orientation. The masses of poorly developed fibers remained embedded in amorphous, PAS-positive ground substance, which did not stain metachromatically. The clear interfibrillary spaces noted between the fibers of the control tissue were obscured by persistence of amorphous ground substance. The fibers present stained less red than those in the control with Van Gieson's picrofuchsin stain.

The impairment of fiber formation varied somewhat and was apparently dependent upon maintenance of a constant AAN level during the postimplantation period. At the

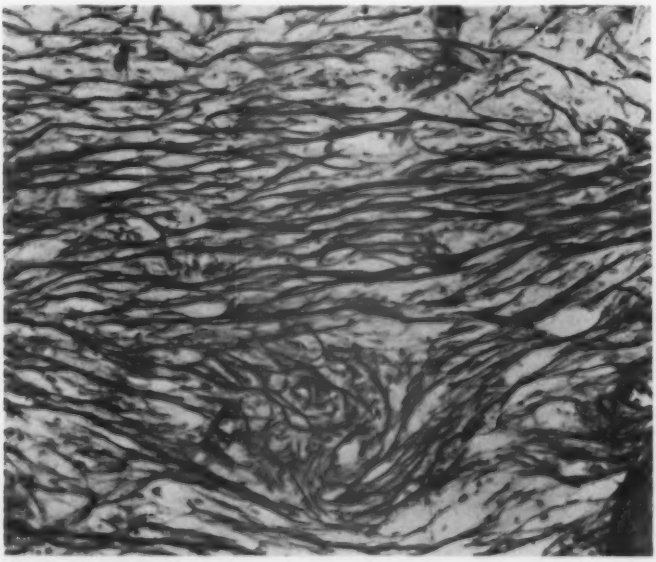


Fig. 5.—Control animal at 14 days. Note well-formed parallel fibers. Snook reticulum stain; reduced to 85% of mag. × 225.

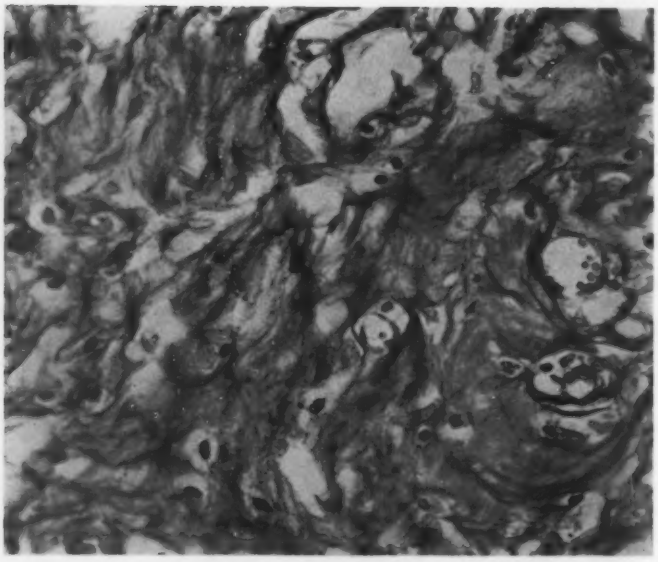


Fig. 6.—AAN-treated animal (25 mg. per day) at 14 days. Note abundant ground substance, numerous vascular channels, and poorly formed fibers. Snook reticulum stain; reduced to 85% of mag. ×225.

AAN dosage used fiber formation was retarded but not fully inhibited. Thus, there was less inhibition of fiber production in the animals on small doses of AAN and after prolonged periods following implantation. Figures 3, 4, 5, and 6 show typical histologic findings in control animals and in the animals given AAN (25 mg. per day). The histologic findings in the semistarvation group did not differ from the controls.

No obvious changes in the appearance of fibroblasts in the AAN group could be detected if changes due to various stages of maturation were taken into account. It is significant, however, that no evidence of any excessive fibroblastic proliferation was seen in any of the sponges removed from the AAN-treated animals. Large mononuclear cells, probably histiocytes, with intracytoplasmic PAS-positive granules, were present in the sponges from both control and experimental animals. Mast cells were scarce, and no difference in their number, granulation, or metachromasia was evident between control and experimental animals. A large number of polymorphonuclear leukocytes was also present in both groups and showed similar karyolytic and karyorrhectic changes. Furthermore, the fibrin which formed in the spaces of the sponges appeared the same in control and in treated animals. In both groups, it served as supporting latticework for the ingrowing fibroblasts, and there was no evidence that it participated in fiber formation in any other way.

Chemical Observations.—The sponge-tissue hydrolysates yielded values of hydroxyproline and hexosamine in control animals which were in the main similar to those found by Dunphy and Udupa in the healing wounds of mature rats.[3] The values obtained in our experiments are shown in Figure 7. The progressive increase of hydroxyproline correlated closely with the new formation and maturation of fibrous connective tissue seen microscopically.

The progressive increase in hydroxyproline was inhibited slightly in animals given 25 mg. of AAN daily. In animals on 50 mg. of AAN, the hydroxyproline values were much lower than those in the control animals. This decrease was evident in all experimental stages. It was appreciable by the third day and was still present on the 25th day after implantation. It was most apparent in the 5- and 10-day studies. Animals on semistarvation whose weight level was kept comparable with the animals of

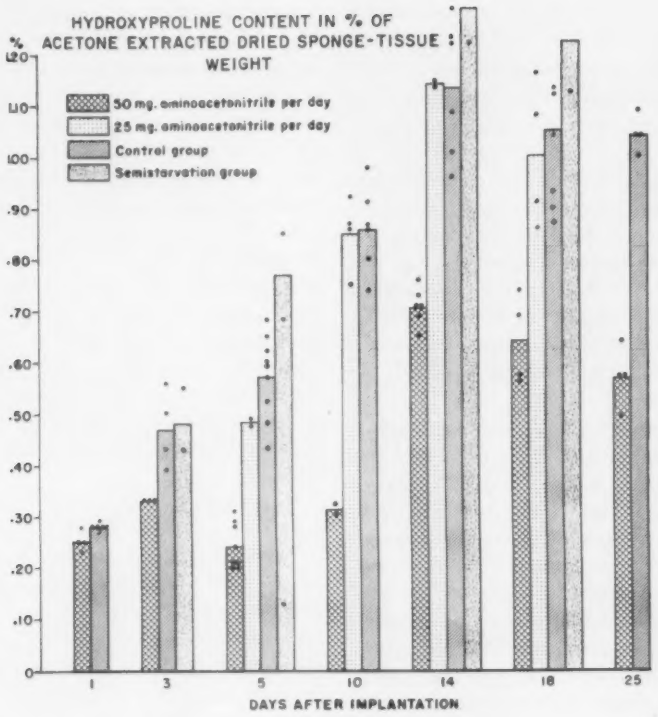


Figure 7

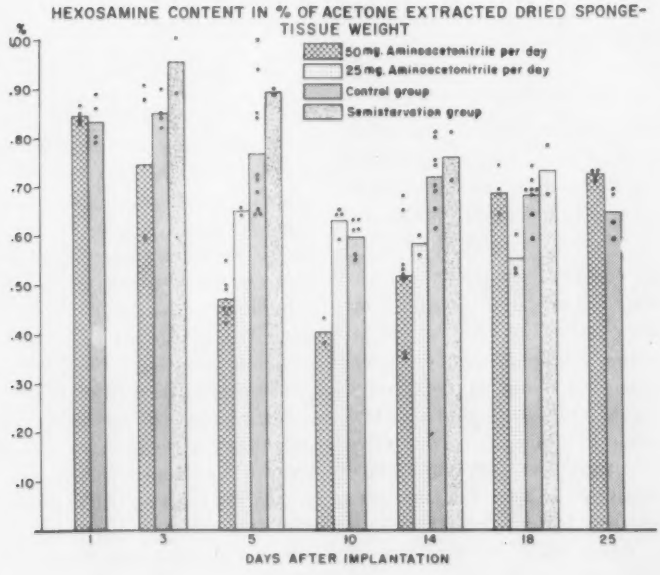


Figure 8

the 50 mg. of AAN group showed slightly lower values in the first few days after implantation but exceeded somewhat normal control values on the 14th and 18th days.

The hexosamine values, shown in Figure 8, decreased somewhat during the first 10 days after implantation in the control group. In animals on 25 mg. of AAN, hexosamine values paralleled those of the control but showed lower levels. In animals kept on 50 mg. of AAN daily, a rapid decline of the hexosamine content was present initially, but in later stages hexosamine values approached and even exceeded those of the control.

For interpretation of the hexosamine values, estimation of the plasma levels was thought important. Control assays of sponges filled with plasma yielded hexosamine values which approached closely those found 24 hours after implantation. Thus, the hexosamine values reflected a composite of several factors: the initial plasma hexosamine level, the hexosamine present in the newly forming ground substance, and the utilization of hexosamine in the developing connective tissue. It was felt that the early greater lowering of hexosamine in the AAN sponges probably represented mainly the retardation of connective tissue growth.

Comment

The foregoing observations are in agreement with the hypothesis that the essential changes of the mesodermal tissue in lathyrism are based on inadequate formation and maturation of connective tissue fibers.

The changes observed are quantitative as well as qualitative and can be roughly divided into two overlapping phases: one in which connective tissue formation is retarded generally and filling of the sponge lags behind that of the control, and a second phase, in which the immature connective tissue fails to mature. Thus, the initial fibrogenesis is relatively delayed but not prevented. Fibrils are formed but remain at an immature stage and formation of strength-bearing collagen fibers seems to be arrested, or at least slowed down considerably. Morphologically, this is evidenced by a poorly oriented feltwork of fragile and delicate fibrils, as seen in the newly formed connective tissue of the implanted sponges. Chemically, the faulty development of connective tissue is reflected in the low hydroxyproline content of the newly formed connective tissue. Although the delay may be due partly to a nonspecific toxic action of the AAN, as suggested by the weight loss, accumulated data in the literature indicate that growing mesodermal tissues are peculiarly susceptible in lathyrism. The failure of the newly formed connective tissue to develop normally in the polyvinyl sponges, thus, is in accord with previous studies on lathyrism.

Excessive fibroblastic proliferation, reported as a constant and prominent feature in both the skeletal and the aortic lesions,[2,11,15-17] was also present in the later stage of wound healing in the rat.[5] However, in the present investigation no such increase in the number of fibroblasts could be observed. It is felt likely that the absence of excessive fibroblastic proliferation in the implanted sponges is due to relative lack of mechanical stress and that excessive fibroplasia represents a reparative phenomenon rather than a primary response to lathyrism.

Summary

Samples of newly formed connective tissue were obtained in lathyritic and control animals by implanting small polyvinyl (Ivalon) sponges into the subcutaneous tissue of 69 albino rats. The sponge samples were removed at various periods of the experiment, ranging from 24 hours to 25 days after implantation. Experimental lathyrism was induced by daily administration of aminoacetonitrile hydrosulfate.

Retardation of connective tissue growth and faulty formation of connective tissue fibers was evident histologically. Low values of hydroxyproline corresponded with the inhibition of normal connective tissue formation. Hexosamine values were initially decreased but rose to normal later and

could not be correlated with connective tissue growth and maturation.

Excessive proliferation of fibroblasts, described in most lesions in experimental lathyrism, was not observed in connective tissue formed in the sponge.

Normal connective tissue growth in control animals on a semistarvation diet indicated that weight loss per se was not contributory to the defective formation of fibrous connective tissue in the lathyritic animals.

We are indebted to Dr. Stanley Wawzonek, of the Department of Biochemistry, the State University of Iowa, for supplying aminoacetonitrile hydrosulfate.

Department of Pathology, State University of Iowa.

Addendum

Since this manuscript was submitted for publication, a similar study has been reported (Hurley, J. V., and Ham, Kathryn N.: The Nature of the Connective Tissue Defect Produced by the Aminonitriles, Brit. J. Exper. Path. 40:216, 1959).

REFERENCES

1. Boucek, R. J., and Noble, N. L.: Connective Tissue: A Technique for Its Isolation and Study, A.M.A. Arch. Path. 59:553, 1955.

2. Dasler, W.: Experimental Lathyrism, Chicago M. School Quart. 18:1, 1957.

3. Dunphy, J. E., and Udupa, K. N.: Chemical and Histochemical Sequences in the Normal Healing of Wounds, New England J. Med. 253: 847, 1955.

4. Elson, L. A., and Morgan, W. T. J.: Determination of Hexosamine, in Methods of Biochemical Analysis, edited by D. Glick, New York, Interscience Publishers, Inc., 1955, Vol. 2, p. 202.

5. Enzinger, F. M., and Warner, E. D.: Wound Healing in Experimental Lathyrism, Lab. Invest. 6:251, 1957.

6. Follis, R. H., Jr., and Tousimis, A. J.: Experimental Lathyrism in the Rat: Nature of Defect in Epiphyseal Cartilage, Proc. Soc. Exper. Biol. & Med. 98:843, 1958.

7. Hamre, C. J., and Yeager, V. L.: Influence of Muscle Section on Exostoses of Lathyric Rats, A.M.A. Arch. Path. 64:426, 1957.

8. Mielke, J. E.; Lalich, J. J., and Angevine, D. M.: Influence of β-Aminopropionitrile upon Development of Connective Tissue in Croton Oil Pouches, Proc. Soc. Exper. Biol. & Med. 94:673, 1957.

9. Neuman, R. E., and Logan, M. A.: The Determination of Hydroxyproline, J. Biol. Chem. 184:299, 1950.

10. Ponseti, I. V., and Baird, W. A.: Scoliosis and Dissecting Aneurysm of the Aorta in Rats Fed with Lathyrus Odoratus Seeds, Am. J. Path. 28:1059, 1952.

11. Ponseti, I. V.: Lesions of the Skeleton and of Other Mesodermal Tissues in Rats Fed Sweet-Pea (Lathyrus Odoratus) Seeds, J. Bone & Joint Surg. 36A:1031, 1954.

12. Ponseti, I. V.; Wawzonek, S.; Shepard, R. S.; Evans, T. C., and Sterns, G.: Further Studies on Lathyrism in the Rat, Proc. Soc. Exper. Biol. & Med. 92:366, 1956.

13. Selye, H.: Lathyrism, Rev. Canad. Biol. 16:1, 1957.

14. Stamler, F. W.: Reproduction in Rats Fed Lathyrus Peas or Aminonitriles, Proc. Soc. Exper. Biol. & Med. 90:294, 1955.

15. Walker, D. G.: Elastic Fiber Alterations in Rats Treated with Lathyrus Odoratus, A.M.A. Arch. Path. 64:434, 1957.

16. Wirtschafter, Z. T.: Acid Mucopolysaccharides in the Histopathogenesis of the Aortic Aneurysms in the Lathyrus-Fed Rat, A.M.A. Arch. Path. 64:577, 1957.

17. Yeager, V. L., and Hamre, C. J.: Histology of Lathyrus-Induced Exostoses of Rats: The Initial Changes at Tendon-Bone Junction, A.M.A. Arch. Path. 64:171, 1957.

Brain Lesions in Chronic Alcoholism

MATTHEW J. G. LYNCH, M.D., M.R.C.P. (Lond.), Sudbury, Ont., Canada
With the Technical Assistance of Mr. Peter Hills, S.T., R.T.

The histopathologic study of the nervous system in chronic alcoholism has shown a peculiar, though understandable, selective emphasis. Perhaps Wernicke may be said to have led the search for the much-sought specific lesion when he described "superior hemorrhagic polioencephalitis" in 1881.[1] Much weight was placed also on the atrophic changes in the corpora mammillaria, as described by Gudden.[2] The Wernicke-Gudden lesions were, however, subsequently shown not to be specific for alcoholism.[3-5] Indeed, Wernicke's third case was one of wasting due to pyloric stricture. Marchiafava and Bignami's [6] necrotizing degeneration of the corpus callosum was for long believed to be both racially specific for Italians and exclusively associated with habituation to crude Italian red wine. This dual specificity was disproved by later workers.[7]

In 1899 Bonhoeffer [8] described degenerative changes in the granular layer and dentate nuclei of the cerebellum, and in 1905 Thomas [9] drew attention to an almost constant loss of Purkinje cells in chronic alcoholics. After a long period of dormancy, emphasis in recent decades has increasingly been placed on degenerative cerebellar changes. This reemphasis on cerebellar lesions began when Stender and Lüthy [10] noted a loosening of the nuclear layer and a great loss of Purkinje cells in a man who, while he was a heavy drinker, developed signs and symptoms of cerebellar ataxia and general muscle weakness, and died at the age of 76, having spent the last 16 years of his life as an invalid in an almshouse. Lhermitte and his co-workers [11] produced similar cerebellar degeneration by the administration of alcohol to experimental animals. In his recent study of the brains of 42 alcoholics, Neubürger [12] found the most impressive changes in the cerebellar cortex—in the form of "selective degeneration of the granular layer," which was "only rarely accompanied by serious alterations or loss of Purkinje cells."

In a monograph that is among the best reviews available on the whole subject of the histopathology of alcoholism, Courville [13] points out that the changes described in the brains of persons dying in delirium tremens have been nonspecific, and often not clearly defined from those of associated pellagra. Korsakoff's first case of alcoholic psychosis subjected to histopathologic examination revealed neuroglial proliferation, together with thickening of the walls of cortical blood vessels.[14] Other cortical lesions were described in the literature for the various clinical pictures [13]; yet such alterations never quite gained acceptance as sufficient to explain the illnesses. To quote Neubürger [12]: "Such changes, however, almost always have proved to be rather monotonous and nonspecific." Gamper,[15] in a postmortem study of nine male and seven female chronic alcoholic patients with Korsakoff's psychosis, was unable to find any characteristic lesions in the cortices. The most constant alterations were noted by this author to lie in the corpora mammillaria, brain stem, and basal ganglia, and he concluded that these were the loci of the lesions causing the specific psychosis. Gamper was unable to find definite changes of any type in two cases of delirium tremens. Mott,[16] in 1910, and Carmichael and Stern,[17] in 1931, described lipid pigment deposits in Betz and

Submitted for publication June 9, 1959.

Department of Pathology, Sudbury General Hospital of the Immaculate Heart of Mary.

A portion of the technical work was supported by a grant received from the Alcoholism Research Foundation of Ontario.

other nerve cells, and the latter authors noted the presence of this pigment in most cortical nerve cells and in vascular walls. Marchand,[18] in 14 cases of delirium tremens, found leptomeningeal thickening and congestion, atrophy of pyramidal cells, and perivascular hemorrhages. Courville's findings[13] are broadly similar to those of Marchand. The former author stresses patchy loss of intermediate-layer pyramidal cells and describes heavy loss of cortical and ganglion cells also in Korsakoff's psychosis.

It has been intimated that fat embolism may occur in chronic alcoholics and that it derives from rupture of "fat cysts" in the liver.[19] A study conducted in the department of this hospital has indicated the frequency with which fat embolism may be found in material (lungs and brains) from chronic alcoholics, and evidence has been adduced which suggests that peaks of fat embolism may play a significant role in the etiology of delirium tremens.[20] It was therefore decided to investigate the brains of a series of alcoholic patients by accepted histologic techniques in order to assess, if possible, the part fat embolism may play in the genesis of the lesions revealed.

Materials and Methods

The brains of 11 habitual drinkers with chronic alcoholism of long-standing were studied (Table). Apart from the effects of chronic alcoholism, all patients save one (Case 3) were in average to good general nourishment. In four cases (1, 2, 10, and 11) fat embolism appeared to be the chief and immediate cause of death. In the one case of suicide, death followed within an hour of leaping from the seventh floor of the hospital, fractures of the pelvis and legs being sustained. Five controls were used: three men, aged 32 to 62, and two women, aged 58 and 64; and these were chosen so as to exclude factors other than atherosclerosis, which lead to cerebral degenerations.

The brains were fixed *in toto* or in large slices in 10% buffered neutral formalin (Lilie[21]). After adequate fixation, from each case a minimum of five areas was studied by carefully selected and closely corresponding blocks from both alcoholic and control cases. The areas chosen for study were (1) motor cortex, (2) corpus callosum, (3) basal ganglia (hypothalamus and mammillary bodies, and portions of thalamus and of substantia nigra); (4) cerebellum (including cortex and part of dentate nucleus), and (5) lower medulla-upper cervical cord. In addition, in several cases blocks from the corona radiata, optic radiation, and pons were also studied.

The following staining techniques were employed: (1) routine hematoxylin-eosin method; (2) oil red O staining of frozen sections for fat[22]; (3) polychrome methylene blue method of Roussy and Lhermitte, as modified by Carleton and Drury[23]; (4) Gordon and Sweet's method

Alcoholic Cases Investigated

Case No.	Age, Yr.	Sex	General Nourishment	Clinical State	Liver: Fat & Cirrhosis 0-4+	Brain Changes, 0-4+ *		Fat Embolism †
						Gross	Micro.	
1	40	M	Obese	Died in D.T.'s	Enlarged: cirrhosis, 3; fat 4	2-3	2-3	Heavy
2	46	M	Obese	Died in D.T.'s	Enlarged: cirrhosis, 1; fat 4	3	3	Heavy
3	56	F	Average	Pulmon. & ileocecal Tb.	Enlarged: cirrhosis, 0; fat, 4	2	2	Mild
4	33	F	Average	Suicide in D.T.'s	Enlarged: cirrhosis, 0; fat, 4	1-2	2	Heavy (probably mainly traumatic)
5	59	M	Average	Hepatic coma	2,000 gm.; cirrhosis, 4; fat, 3	2-3	3	Mild
6	46	F	Obese	Bronchopneumonia	1,840 gm.; fat, 4; cirrhosis, 2-3	2	2	Moderate
7	62	M	Obese	Hemorrhage, esophageal varices	Reduced: cirrhosis, 3; fat, 3	3-4	4	Moderate
8	47	M	Obese	Hemorrhage, esophageal varices	Reduced: cirrhosis, 3; fat, 3	4	4	Moderate
9	58	M	Obese	Hemorrhage, esophageal varices	1,320 gm.; cirrhosis, 4; fat 2-2½	4	4	Mild
10	51	M	Average	Died in D.T.'s	3,200 gm.; cirrhosis, 0; fat, 4	3	3	Heavy
11	56	M	Obese	Died in D.T.'s	3,700 gm.; cirrhosis, ½; fat, 4	4	4	Heavy

* Gross brain changes were rated on the basis of degrees and combinations of atrophy and shrinkage (cortical and cerebellar atrophy, and enlargement of ventricles), pinpoint porencephaly (porosities), petechiae, and leptomeningeal thickening. Microscopic ratings were based on perivascular hemorrhages and macrophages, nerve-cell loss (chiefly motor cortex and cerebellum), and myelin loss.

† "Heavy" indicates more than 20 emboli per square centimeter of lung section.

for reticulin fibers (Clayden[23]); (5) Gridley's reticulin stain[24]; (6) Weil's method for myelin sheath[25]; (7) Busch's method for degenerated myelin[26]; (8) Weil and Davenport's method for glial cells[27]; (9) Roger's modification of Foot's method for nerve fibers and neurofibrils,[28] and (10) Luxol fast blue-PAS (periodic acid-Schiff)-hematoxylin for myelin.[9]

Results

Gross Findings.—There is little to add to the gross descriptions in the literature.[12,13] One feature that has received little emphasis is the striking abundance in these brains—chiefly in the white matter and the lenticular nucleus and thalamus—of tiny pinpoint- to pinhead-sized holes. Almost all of these tiny porosities surround small blood vessels. Careful search often revealed fine petechiae in small to moderate numbers, being most numerous in the cases showing heavy fat embolism. Such petechiae were noted with greatest frequency in the septum pellucidum, internal capsule, corona radiata, optic radiation, corpus callosum, and ependyma of the ventricular system. In no case were the findings characteristic of the Wernicke or Marchiafava-Bignami entities seen. Cortical cerebral atrophy was present to some degree in all instances, but it appeared to be diffuse, and I was unable to satisfy myself that it was most prominent or was confined to the frontal lobes, as some observers have remarked.[13] Leptomeningeal thickening was noted in all cases and usually displayed patchy, focal intensifications. Cerebellar atrophy was usually detectable grossly—not by over-all reduction in size but by thinning of the individual folia and a peculiarly firm consistency of the whole hindbrain. Atheromatous changes in the blood vessels were, if anything, less than that usually seen in the corresponding age groups of nonalcoholics.

Microscopic Findings.—Increased vascularity (Figs. 1 and 2) was demonstrable in all areas of all brains from the alcoholic group, and was shown to best advantage by Gordon and Sweet's method for reticulin. Loss of myelin was easily detectable in certain areas, e.g., the outer cortical layers, where the myelin bundles are shown in relief and contrast with other structures. Both Weil's and the Luxol fast blue-PAS-hematoxylin methods revealed a striking loss of

Fig. 1.—Vasculature of normal corpus callosum. Gordon and Sweet's stain; × 100.

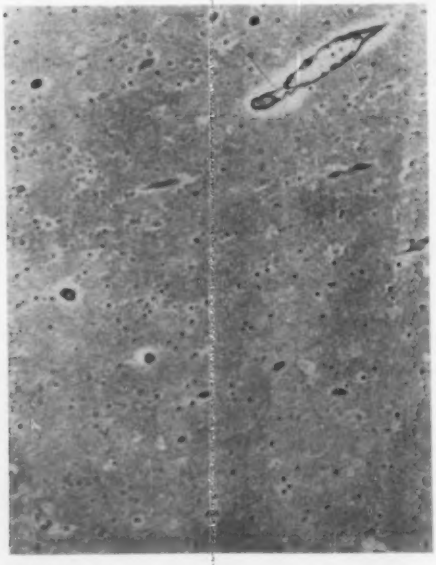


Fig. 2 (Case 7).—Increased vascularity of corpus callosum in chronic alcoholism. Gordon and Sweet's stain; × 100.

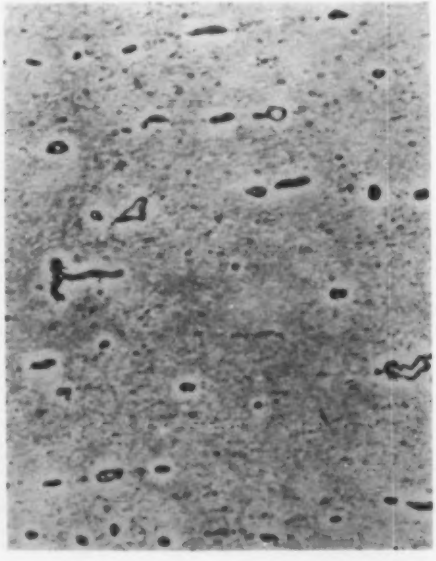


Fig. 3.—Normal outer motor cortex. The myelinated radial fibers are well shown in the lower half. Weil's stain; reduced to 59% of mag. × 40.

Fig. 4 (Case 9).—Outer motor cortex of chronic alcoholic. There is a very considerable loss of myelin. Weil's stain; reduced to 66% of mag. × 40.

the outer, radial myelin fibers of the cerebral cortex (Figs. 3 and 4). Similar degrees of myelin-sheath loss in heavily myelinated areas, e.g., corona radiata and brain stem, are more difficult to assess and demonstrate, but careful comparison with controls often suggests some reduction of myelin in these areas also. In the cerebellum myelin loss is a very striking feature in the axes of the folia and in and around the dentate nucleus (Figs. 10-13).

Quantitative comparison of sections of motor cortex from corresponding areas of brains of controls and of alcoholics revealed a 20% to 40% reduction in the number of pyramidal cells in the latter. In addition, almost all of the remaining cells displayed considerable shrinkage, and nuclear pyknosis was also a common feature (Figs. 5 and 6). With Foot's method, small foci of nerve-fiber loss—usually round to wedge-shaped—were occasionally seen in the lower cortex and the junction of the white and the cortical gray matter (Fig. 7). Local glial increase was less frequently found in the same location and was rarely seen elsewhere (Figs. 8 and 9).

In the cerebellum, loosening and pallor of the granular layer was constant (Fig. 2). Quantitative comparisons uncovered a 20% to 35% reduction in the number of Purkinje cells, while the cells of the dentate nuclei showed a 25% to 50% loss (Figs. 12 and 13). Similarly, losses as severe as 50% were noted in the anterior horn cells of the upper cervical cord. Reduction in numbers of ganglion cells in the basal ganglia and pontile and olivary nuclei was also noted, but, because of the complexity of structures in these areas, no estimate of the degree of cell loss was attempted.

Constantly seen in all alcoholic cases were such well-recognized changes as leptomeningeal thickenings, increase in the Virchow-Robin spaces (Figs. 7, 8 and 15), abundant

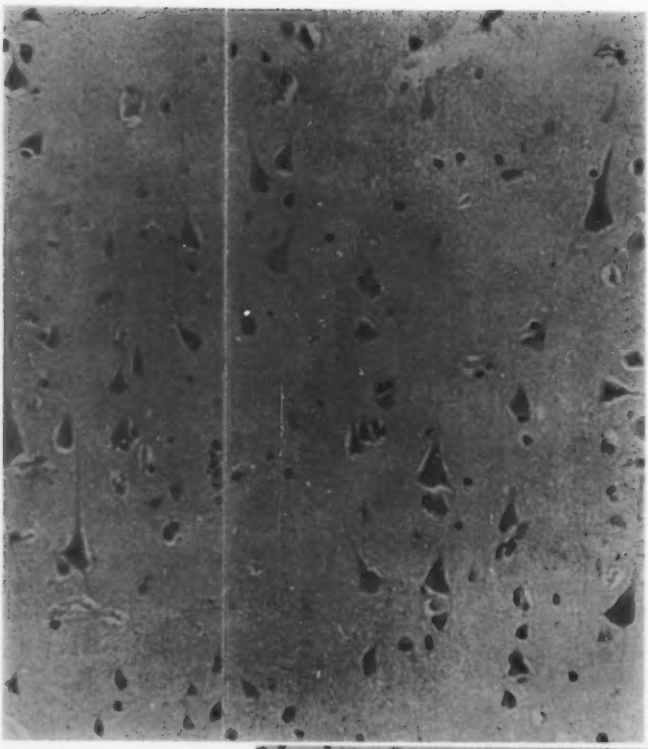


Fig. 5.—Normal motor cortex showing abundant, well-preserved pyramidal cells, from control, aged 62. Hematoxylin and eosin; × 180.

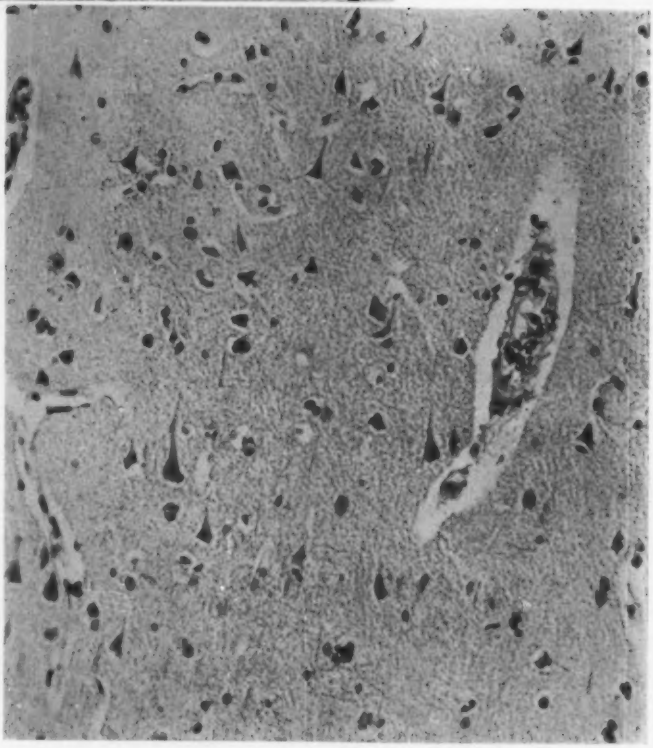


Fig. 6 (Case 9).—Motor cortex of chronic alcoholic. There are increased vascularity, reduction in number of pyramidal cells, and shrinkage of the cells remaining. Compare with Figure 5. Hematoxylin-eosin stain; × 180.

Fig. 7 (Case 7).—Motor cortex of chronic alcoholic. Two foci of nerve-fiber loss; also widening of Virchow-Robin spaces. Foot's stain; reduced to 61% of mag. × 60.

Fig. 8 (Case 4).—Subcortical white matter in case of chronic alcoholism. Local glial proliferation. Weil and Davenport's stain; × 100.

perivascular, lipid-laden macrophages,[20] and small perivascular hemorrhages. The last-mentioned lesions were most commonly seen in the corpus callosum (Fig. 14). Collections of scavenger cells were often in evidence in the leptomeninges,[20] and various degrees of organization of such exudates suggested that this may be one cause of thickening of the leptomeninges.

All of the cases of alcoholism in the present investigation displayed fat embolism in

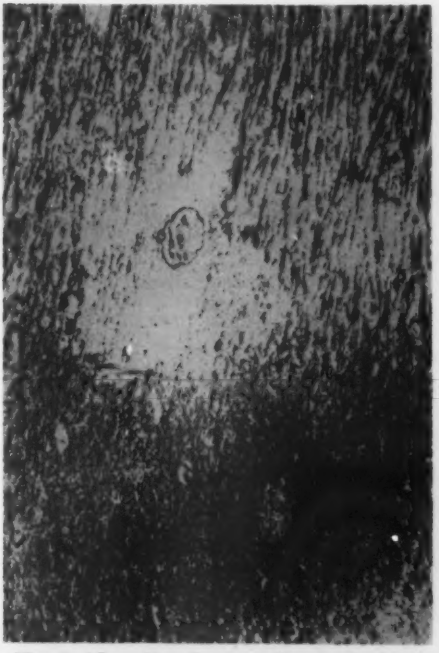


Fig. 9 (Case 8).—Corpus callosum of chronic alcoholism showing a perivascular focus of nerve-fiber loss and an area of gliosis. Foot's stain; reduced to 61% of mag. × 60.

sections of lungs and brain. In four cases this embolism was, in relation to the average for alcoholics,[20] heavy, and ranged from 30 to 80 fat emboli per square centimeter of 30μ lung sections. Fat emboli were seen in great abundance in the choroid plexuses (Fig. 16). Care and experience provided satisfactory frozen sections of these structures after simple formalin fixation. The small fragments were most easily manipu-

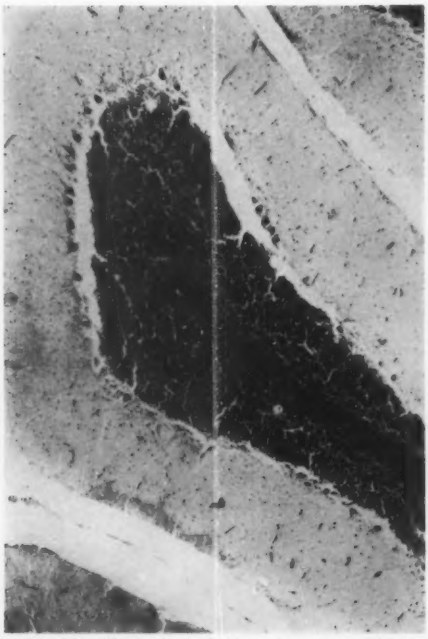


Fig. 10.—Normal cerebellum from control, aged 58 years. Note abundant Purkinje cells, dense granular layer, and central axial myelin, and compare with Figure 11. Weil's stain; reduced to 61% of mag. × 60.

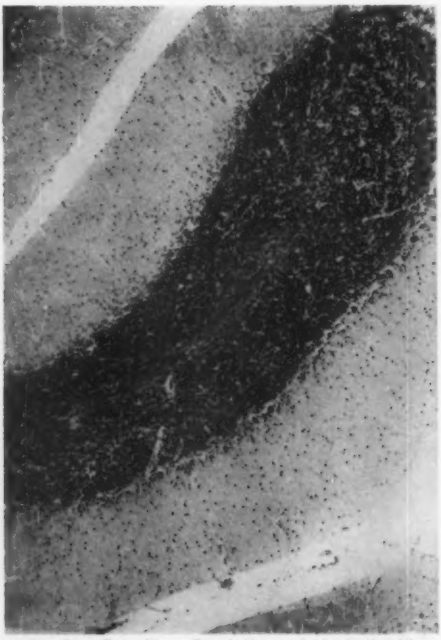


Fig. 11 (Case 7).—Cerebellum of alcoholic. Note reduction of Purkinje cells, loosening of granular layer, and great loss of axial myelin. Weil's stain; reduced to 61% of mag. × 60.

Fig. 12.—Normal dentate nucleus of control, aged 32. Luxol fast blue-PAS-hematoxylin stain. Compare with Figure 13. Reduced to 59% of mag. × 100.

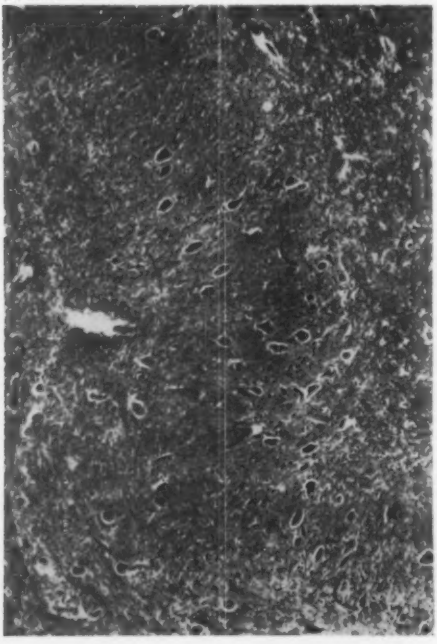
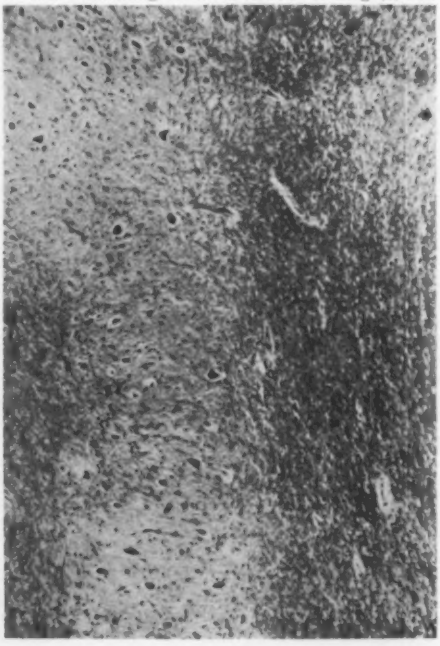


Fig. 13 (Case 10).—Dentate nucleus from chronic alcoholic. Luxol fast blue-PAS-hematoxylin stain. Compare with Figure 12, and note loss of myelin and of ganglion cells, with shrinkage of those remaining. Reduced to 66% of mag. × 100.



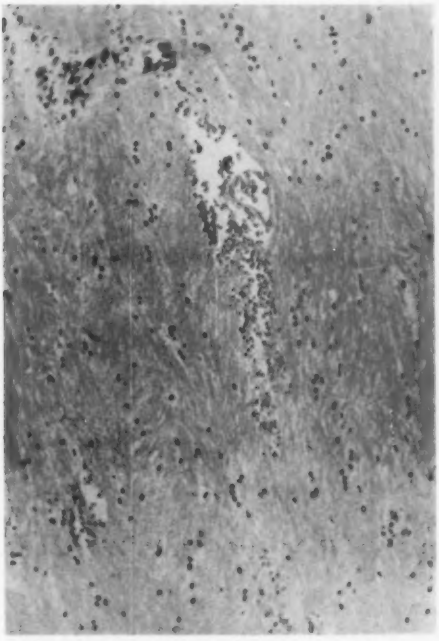


Fig. 14 (Case 8).—Corpus callosum from chronic alcoholic. Four small perivascular hemorrhages are seen. Hematoxylin-eosin stain; reduced to 66% of mag. × 180.

lated for staining by use of a broad-mouth Pasteur pipette, the actual staining being done in a small test tube or centrifuge tube. In these tubes the fragments gradually settle out, allowing the supernatant stain solution to be pipetted off with ease. After washing, the fragments were flooded onto slides and excess water was removed by use of filter paper applied edge-on.

I have noticed that the number of fat emboli demonstrable in the brains of alcoholic patients falls somewhat short of what might be expected from the degrees of embolization in the lungs of these cases, though examination of the choroid plexus was always less disappointing. Experience suggested that a number of emboli were lost from brain sections during manipulation and staining. To test this suspicion, duplicate blocks were taken from four areas in each of two brains. One set was submitted to frozen sectioning after formalin fixation. From each area a contiguous duplicate block was impregnated with and embedded in gelatin, using Aschoff's modification.[22] After hardening of the gelatin blocks, frozen sections were cut and stained in the usual manner. Comparison of the two sets revealed that the sections prepared from the gelatin blocks were of better quality and contained from two to four times as many fat emboli as were found in sections from the nongelatin blocks of the same (immediately contiguous) areas of brain. The gelatin method is strongly recommended also for preparing frozen sections of choroid plexus.

Fig. 15 (Case 3).—Cerebrum of chronic alcoholic, showing enormous widening of the Virchow-Robin space and perivascular macrophages. Hematoxylin-eosin stain; reduced to 63% of mag. × 60.

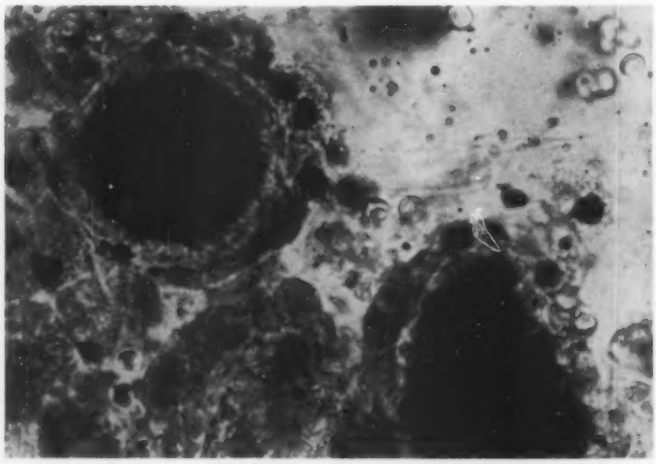


Fig. 16 (Case 11).—Choroid plexus from chronic alcoholic. Frozen section; oil red O stain, showing two capillaries plugged with fat emboli; reduced to 63% of mag. × 1,260.

Comment

The selective lesions of the brain stem described by earlier workers have been shown to be largely due to associated vitamin deficiencies,[3-5] and are now rarely found in uncomplicated alcoholism.[12] It is understandable that these striking alterations were the focal points of attention for many investigators and that the more ubiquitous, but subtler, changes attracted little more than passing comment. Equally, it is not surprising that emphasis today has shifted to the cerebellum, for it is precisely in such a clearly demarcated and structurally contrasting part of the brain that alterations are most striking and easily detected. However, it is apparent to all that cerebellar lesions, no matter how severe, are insufficient to account for the protean impairment of cerebral function so commonly seen in chronic alcoholics. A rough quantitation—and any estimate must necessarily be somewhat crude—of the degree of loss of neurons from such vital sites and structures as the cerebral cortex, dentate nuclei, Purkinje cells, and anterior horn cells offers substantial reason for impairment of cerebral and neuromuscular functions. If we take but the simplest view of the nervous system, viz., that of a multimillion-unit electronic computer, activator, and integrator, it is obvious that the loss of some 15% to 50% of its "transistors" will lead to detectable impairment in many ranges of its functional endeavors.

The difficulty in demonstrating changes, degenerations, and loss of components in such complex, crowded, and relatively contrast-lacking areas as the cortical gray matter and basal ganglia or in such monotonously homogeneous sites as the white matter is not surprising. It is my opinion that such changes can be unequivocally demonstrated by application of careful technique and painstaking comparison with corresponding areas from controls. Indeed, such degenerations have been described by many investigators,[13,14,16-18] but have seemingly escaped the attention they deserve because of lack of easy and convincing histopathologic demonstrability and because they have been overshadowed by more dramatic, though probably less specific, alterations. In the present study the diffuseness of the degenerative changes and the lack of specific localization have been impressive. Alterations were not striking or easily demonstrable only where uniformity or density of structure precluded their easy detection. If a wedge of myelinated fibers has been destroyed, this loss can be detected by special stains if of recent occurrence, but if of long standing, collapse-condensation may hide the process altogether—or leave only a tiny glial scar—and in my experience glial proliferation is not a common accom-

paniment of alcohol-induced degenerations. It is noteworthy that in the material investigated changes in such classical sites as the corpora mammillaria and brain stem have been relatively insignificant.

In the present state of our knowledge, one can only speculate as to the etiology of the degenerative processes in the brains of chronic alcoholics. Courville [13] remarks in regard to cortical degeneration: "Because these areas of chronic cell loss occur in what appears to be avascular foci, it is presumed that some interference with capillary blood supply lies at the root of the process." Courville cites Leevy and his associates [25] and other workers as remarking on the association of liver disease (fatty infiltration) and delirium tremens. The importance of the role played by hepatogenic fat embolism in the causation of delirium tremens has been suggested by a previous investigation.[20] Many of the lesions in the brains of chronic alcoholics have, as Courville [13] remarks, a pericapillary distribution and could be due to ischemia produced by blocking (probably temporary) of the vessel by a plastic lipid embolus. Though fat embolism in chronic alcoholics, by comparison with that seen in trauma cases, is light, nevertheless, frequently repeated episodes of embolization over a period of several years may reasonably be expected to add up to a rather considerable and diffuse loss of nerve tissue components.

The association between fatty liver and delirium tremens has already been remarked upon, and some personal observations in this connection may be worthy of record. It has been my impression that alcoholism in the well-nourished presents quite a different problem from that seen in the case of the poorly nourished or in those existing on a liminal diet. The well-nourished develop fatty liver, delirium tremens, and cirrhosis if the alcoholic habit is continued. On the other hand, though I have had occasion to perform many autopsies on Indians who have been chronically addicted to alcohol—"goof" and "wine-hounds" of humble status—I have never found much fatty infiltration (usually none) and have only rarely found Laennec's cirrhosis in the livers of such cases. Furthermore, brain changes have been much less evident in these poorly nourished cases—with one exception; i.e., loosening of the granular layer of the cerebellum has been commonly found when other changes have been minimal. It is also my impression that delirium tremens is distinctly rare in such cases. However, it must be made clear that these opinions have not been subjected to critical study or analysis, as sufficient material was not available from these cases for inclusion in the present investigation.

Notwithstanding all this evidence, it would be both premature and rash to attempt to explain all, or even most, aspects of alcoholic cerebral degeneration on the basis of fat embolism. The problem is a most complex one, and attention has already been drawn to the possibility that at least one type of degeneration, viz., that of the granular layer of the cerebellum, may be entirely unconnected with fat embolism. Lipid embolization from fatty livers is probably a factor of some considerable importance in the genesis of alcoholic brain lesions; but there are others that are potentially, at least, of equal moment, e.g., repeated toxic and anoxic factors, as well as relative or absolute deficiencies of metabolites and vitamins essential for nervous tissue function and survival.

Summary

The brains of 11 chronic alcoholic patients were investigated by a variety of standard histologic techniques, sections being compared with similarly treated ones from closely corresponding areas of five controls. Constant findings were as follows: 1. Gross: generalized mild to moderate shrinkage, accompanied by enlargement of the ventricular system; leptomeningeal thickening; diffuse thinning or atrophy of cortical gray matter and of cerebellar folia; abundant minute porosities, especially in the white matter and around the internal capsule, and advanced fatty infiltration of livers. 2. Microscopic: increased vascularity; 20%-50% loss of pyramidal, Purkinje,

dentate nucleus, and anterior horn cells; loss of cells from the basal ganglia and the olivary nucleus, etc., evident but not estimated; loss of myelin, which was most easily seen in the outer cortex, axes of the cerebellar folia and in and about the dentate nuclei; widening of Virchow-Robin spaces; perivascular lipid-laden macrophages; scattered, small perivascular hemorrhages (not usually abundant); focal myelin and nerve-fiber loss; shrinkage and pyknosis of many remaining cells; loosening and decreased cellularity of the granular layer of the cerebellum; fat emboli demonstrated in the lungs, choroid plexuses, and brains, and more easily and abundantly found in gelatin-embedded material from the brain.

Repeated episodes of fat embolism over a period of years may be important in the genesis of many of these lesions, though it would appear that loosening of the granular layer of the cerebellum is independent of such embolization. It is my impression that the well-nourished chronic alcoholic suffers more cerebral deterioration and degeneration than the poorly nourished addict does. In the present state of our knowledge, however, no definite conclusions can be reached, but it is apparent that fat embolization is as important as other, less tangible factors.

Department of Pathology, Sudbury General Hospital of the Immaculate Heart of Mary, Paris St.

REFERENCES

1. Wernicke, C.: Lehrbuch der Gehirnkrankheiten, für Aerzte und Studierende, Kassel and Berlin, Theodor Fischer, 1882, Vol. 2, pp. 229-242.

2. Gudden, H.: Klinische und anatomische Beiträge zur Kenntniss der multiplen Alkoholneuritis nebst Bemerkungen über die Regenerationsvorgänge im peripheren Nervensystem, Arch. Psychiat. 28:643-741, 1896.

3. Neubürger, K. T.: Über die nichtalkoholische Wernickesche Krankheit, insbesondere über ihr Vorkommen beim Krebsleiden, Arch. path. Anat. 298:68-86, 1936.

4. Neubürger, K. T.: Wernickesche Krankheit bei chronischer Gastritis: Ein Beitrag zu den Beziehungen zwischen Magen und Gehirn, Ztschr. ges. Neurol. 160:208-225, 1937.

5. Biggart, J. H.: Pathology of the Nervous System: A Student's Introduction, Ed. 2, Edinburgh, E. & S. Livingstone, Ltd., 1949, p. 195.

6. Marchiafava, E., and Bignami, A.: Sopra un alterazione del corpo calloso osservata in soggetti alcoolisti, Riv. pat. nerv. 8:544-549, 1903.

7. Nielsen, J. M., and Courville, C. B.: Central Necrosis of the Corpus Callosum (Marchiafava-Bignami's Disease): Report of a Case in an American Man with Comments on the Pathogenesis of the Disease, Bull. Los Angeles Neurol. Soc. 8:81-88, 1943.

8. Bonhoeffer, K.: Pathologisch-anatomische Untersuchungen an Alkoholdeliranten, Monatsschr. Psychiat. u. Neurol. 5:265-284, 379-387, 1899.

9. Thomas, A.: Atrophie lamellaire des cellulas de Purkinje, Rev. neurol. 13:917-924, 1905.

10. Stender, A., and Lüthy, F.: Über Spätatrophie der Kleinhirnrinde bei chronischem Alkoholismus, Deutsche Ztschr. Nervenh. 117-119: 604-622, 1931.

11. Lhermitte, J.; de Ajuriaguerra, J., and Garnier, J.: Lés lesions du système nerveux dans l'intoxication alcoolique expérimentale, Compt. rend. Soc. biol. 128:286-288, 1938.

12. Neubürger, K. T.: The Changing Neuropathologic Picture of Chronic Alcoholism: Prevailing Involvement of the Cerebellar Granular Layer, A.M.A. Arch. Path. 63:1-6, 1957.

13. Courville, C. B.: Effects of Alcohol on the Nervous System of Man, Los Angeles, San Lucas Press, 1955.

14. Korsakoff, S. S., and Serbski, W.: Ein Fall von polyneuritischer Psychose mit Autopsie, Arch. Psychiat. 23:112-134, 1892.

15. Gamper, E.: Zur Frage der Polioencephalitis haemorrhagica der chronischen Alkoholiker: Anatomische Befunds beim alkoholischen Korsakow und ihre Beziehungen zum klinischen Bild, Deutsche Ztschr. Nervenh. 102:122-129, 1928.

16. Mott, F. W.: The Nervous System in Chronic Alcoholism, Brit. M.J. 2:1403-1408, 1910.

17. Carmichael, E. A., and Stern, R. O.: Korsakoff's syndrome: Its Histopathology, Brain 54: 189-213, 1931.

18. Marchand, L.: Lés lésions du système nerveux, du foie, des reins et de la rate dans le "delirium tremens" des alcooliques, Ann. anat. path. 9:1026-1030, 1932.

19. Hartroft, W. S., and Ridout, J. H.: Pathogenesis of the Cirrhosis Produced by Choline Deficiency: Escape of Lipid from Fatty Hepatic Cysts into the Biliary and Vascular Systems, Am. J. Path. 27:951-989, 1951.

20. Lynch, M. J. G.; Raphael, S. S., and Dixon, T. P.: Fat Embolism in Chronic Alcoholism:

Control Study on Incidence of Fat Embolism, A.M.A. Arch. Path. 67:68-80, 1959.

21. Lillie, R. D.: Histopathologic Technic and Practical Histochemistry, New York, The Blakiston Company (Medical Division of McGraw-Hill Book Company, Inc.) for Normal and Pathological Tissues and the Identification of Parasites, Ed. 3, 1954.

22. Carleton, H. M., and Drury, R. A. B.: Histological Technique, London, Oxford University Press, 1957.

23. Clayden, E. C.: Practical Section Cutting and Staining, Ed. 3, London, J. & A. Churchill, Ltd., 1955.

24. Manual of Histologic and Special Staining Technics, Armed Forces Institute of Pathology, Washington, D.C., 1957.

25. Leevy, C. M.; Patrylo, I., and Doody, W.: Hepatic Abnormalities in Alcoholics with Delirium Tremens, Quart. J. Stud. Alcohol 14:568-575, 1953; cited by Courville.[18]

Correction

In the article entitled "Capillaries of Normal and Diseased Breast" by Herbert Fanger, M.D., and Barbara E. Barker, M.S., appearing in the January Archives, the first sentence of the first paragraph on page 75, should read: "The presence of blood vessels of capillary size in cases of carcinoma has been demonstrated by the Lepehne-Pickworth peroxidase method." This error occurred when a corrected line ending in the same word was inserted at the beginning of a preceding paragraph.

A Papain Digestion Apparatus

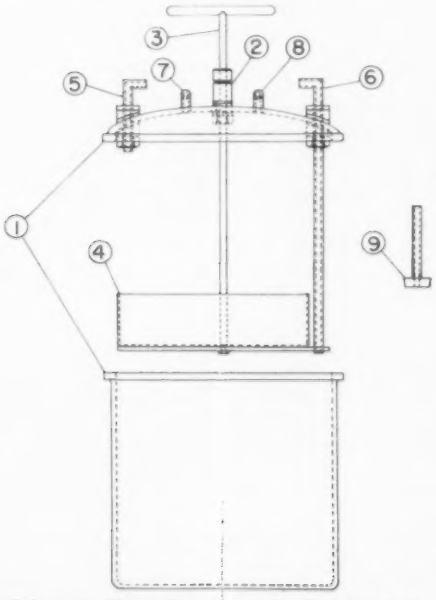
L. SOKOLOFF, M.D.; R. D. LILLIE, M.D., and F. O. ANDERSON, Bethesda, Md.

The preparation of dried bones for anatomical and pathological examination by maceration with papain has technical advantages over other methods in several circumstances, particularly when the specimens are small. During this procedure, however, offensive odors of putrefaction develop. The following apparatus has been found to eliminate this difficulty. Basically it is a closed digestion tank with inflow and outflow connections that allow the noxious materials to be flushed away.

Submitted for publication June 9, 1959.

Present address Dr. Lillie: Department of Pathology, Louisiana State University School of Medicine, New Orleans 12.

From the National Institute of Arthritis and Metabolic Diseases, Laboratory of Pathology and Histochemistry (Drs. Sokoloff and Lillie), and the Division of Research Services, Laboratory Aids Branch (Mr. Anderson) of the National Institutes of Health, U.S. Public Health Service, U.S. Department of Health, Education, and Welfare.



Schematic illustration of papain digestion tank.

Mechanical Features

The design of the apparatus is illustrated in the Figure.

In the interest of expediency and cost, the apparatus was constructed by modifying a 21-quart aluminum pressure canner (*1*).*

Modifications involving the top only are as follows: An "O" ring seal and bearing (*2*) was attached through a hole drilled in the center. Through this was placed a handle (*3*) attached to a stainless-steel tray with a mesh bottom (*4*). This is for holding specimens and for agitation during processing. A water inlet (*5*) and an outlet (*6*) pipe were provided for filling and flushing. The outlet pipe extends to within ⅛ in. of the bottom, so that all residue is flushed out of the chamber. Vacuum (*7*) and pressure (*8*) connections were provided for aiding in filling and flushing. The pressure-regulating valve was removed and the hole plugged. The pressure gauge and blow-out plug were retained as a safety feature.

The inlet pipe was connected to the water supply by rubber tubing through a standard air-gap water fixture, to prevent possible contamination of water source. This fixture was placed close to the ceiling to provide as high a head of water as possible, since an air-gap fixture does not transmit pressure. The outlet pipe was connected to hollow tapered metal sink stopper (*9*) by transparent, heat-resistant Tygon tubing † to prevent the escape of odors when flush-

* Presto Cooker-Canner, No. 21AV; National Presto Industries, Eau Claire, Wis.

† Standard Tygon tubing, Potomac Rubber Co., Inc., Washington, D.C.

ing. Vacuum and pressure connections were made with rubber tubing.

Specifications are available from us.

The tank is installed in an anhydric bacteriologic incubator with inside dimensions 19¼ × 17 × 14½ in.‡ Temperature is maintained at 50 C to decrease odors of bacterial putrefaction, as well as to accelerate digestion.

Operation

The animals are skinned and eviscerated. In large specimens, the bulk of the attached muscle is dissected away. The latter procedure may be facilitated by preliminary autoclaving for 45 to 60 minutes at 15 lb. pressure. The carcass is then placed in a cloth bag § along with an accession number stamped on stainless steel tape.|| Powdered papain is introduced into the bag, the amount varying with the size of the specimen. For a mouse, approximately a teaspoonful (2.5 gm.) is adequate. Several dozen mice can be accommodated at one time by the tank.

Water is introduced through the inlet (*5*) while the air inlet (*8*) is unclamped until the specimens are covered. All tubing is clamped shut during digestion. The digesting material is agitated vigorously once a day by raising and lowering the handle (*3*). The sink stopper is not placed into the outlet of the sink except when the contents of the tank are being flushed out. The time required for completion of the maceration varies with the size of the specimen; for mice, three to four days is sufficient. For larger specimens, it may be 7-10 days. Drainage of the digestate is facilitated by introduction of air under pressure (*8*). The material is then flushed out by a continuous flow of water for 30 to 60 minutes.

Following maceration, the bones are bleached with 30% hydrogen peroxide (Superoxol), rinsed with water, and defatted with acetone.

The apparatus has been employed successfully in the preparation of bones of several species—mice, rats, pigs, birds, and man.

‡ Elconap Type A2, Electric Heat Control Apparatus Co., Newark, N.J.

§ Plain white parts bags, Golden Belt Manufacturing Co., Durham, N.C.

|| Handy Garden Label Embosser, Roover Bros., Inc., Brooklyn.

Summary

A simple apparatus is described for preparing dry bone by maceration with papain, without release of noxious odors.

National Institute of Arthritis and Metabolic Diseases.

News and Comment

ANNOUNCEMENTS

First International Congress of Histochemistry and Cytochemistry.—The First International Congress of Histochemistry and Cytochemistry will be held in Paris, Aug. 28-Sept. 3, 1960. Further details may be obtained from Dr. R. Wegmann, secretary general, 45, Rue des Saint-Peres, Paris 6e.

PERSONAL

Annual Carl V. Weller Lecture.—The Fourth Annual Carl V. Weller Lecture to the Michigan Pathological Society was given at the University of Michigan on Dec. 12, by Dr. Shields Warren, of Boston. Dr. Warren talked on "The Pathology of Ionizing Radiation."

Dr. John R. Carter's Appointment.—Dr. John R. Carter, formerly of the University of Iowa, has been appointed professor of pathology and chairman of the department at the University of Kansas School of Medicine, effective Jan. 1, 1960.

Dr. Hugh G. Grady's Appointment.—Dr. Hugh G. Grady, Director of the Department of Pathology at the Seton Hall College of Medicine, Jersey City, N.J., has been appointed Acting Dean.

SOCIETY NEWS

Fourth National Cancer Conference of the American Cancer Society.—The Fourth National Cancer Conference of the American Cancer Society will be held in Minneapolis, Sept. 13-15, 1960. Further details may be obtained by addressing the American Cancer Society, Inc., Medical Affairs Department, 521 W. 57th St. New York 19.

Fourth International Criminological Congress.—The Fourth International Criminological Congress will be held in The Hague Sept. 5-12, 1960. Further details may be obtained from the Secretariat of the Fourth International Criminological Congress, 14, Burgemeester de Monchyplein, The Hague, Netherlands.

Books

Cancer in Families. By Douglas P. Murphy, M.D., and Helen Abbey, Sc.D. Price, $2.50. Pp. 76. Harvard University Press, Cambridge, Mass., 1959.

This book discusses the results of an extensive field investigation of the families of breast-cancer patients. It is an interesting and detailed account of methods of obtaining, tabulating, and analyzing statistical material concerning cancer. The authors conclude that the data reveal no evidence of any unusual frequency of cancer either of the breast or elsewhere among blood relatives of breast-cancer patients.

It should be pointed out that other statistical studies carried out in different manners have not arrived at similar results. One hesitates to conclude from this study that heredity is or is not involved in the incidence of breast cancer.

The Biology of Myelin. Progress in Neurobiology: IV. Edited by Saul R. Korey, M.D., and John I. Nurnberger, M.D. Price, $9.50. Pp. 426, with 182 illustrations. Paul B. Hoeber, Inc. (Medical Division of Harper & Brothers), 49 E. 33d St., New York 16, 1959.

This book is the fourth volume resulting from a series of symposia on current basic neurological research. The major contributors and participants are eminent in their fields of chemistry, electron microscopy, tissue culture, or neurophysiology. Although myelin is an exclusive phenomenon of the nervous system, its study has general biologic importance, since its structure as shown by the electron microscope is reminiscent of biological membranes elsewhere. The special value and interest of this book relates to the areas of disagreement expressed by workers with technically different approaches to the same problem. This is best illustrated by the differing points of view on myelogenesis between electron microscopists and those studying tissue culture. The extensive discussions of the presentations are lively and generally provocative. This book is not recommended for those who expect ultimate answers to questions about myelin, but can be recommended highly to those who are seeking questions to answer. It is well printed and effectively illustrated.

Medicinal Chemistry. Vol. IV. Edited by F. F. Blicks and R. H. Cox. Price, $12.00. Pp. 334. John Wiley & Sons, Inc., 440 Fourth Ave., New York 16, 1959.

The major emphasis is on organic chemical structure with a short review of the pharmacology of the barbiturates. It is unfortunate that more care was not exercised in the definition of pharmacological terms used, i.e., antiepileptic, sedative, hypnotic, analgesic, and anesthetic. The growing and important problem of addiction is reviewed briefly. The inclusion of strychnine as an antidote for barbiturate poisoning is questionable, while the modern treatment of maintaining adequate respiratory exchange is, unfortunately, not mentioned.

The authors have reviewed the literature of barbituric acid derivatives and have compiled extensive tables of structure modifications together with references. Certain generalizations are drawn regarding the relationship between pharmacological activity and molecular structure with the caution that structural analogy can be very misleading and that there is no fundamental basis for the prediction of hypnotic action in the barbituric acid series. This book will have definite value for those interested in the synthesis of new barbiturate derivatives.

Surgical Pathology. Second Edition. By Lauren V. Ackerman, M.D., with Harvey R. Butcher Jr., M.D. Price, $15.00. Pp. 1,096, with 1,114 figures. The C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, 1959.

The bulk of most pathologists' tissue work is surgical specimens; yet only recently has much recognition been given to this subspecialty of pathology. In contrast to the large number of general pathology texts, only a few books have appeared concerned with pathology as it applies to surgical material. One of the first books to consider this field was Dr. Ackerman's "Surgical Pathology," published in 1953. Since he continues to be one of the foremost surgical pathologists in this country, it is fortunate that he has now chosen to revise and expand his book. In this edition he has enlisted the aid of his surgical colleague, Dr. Harvey R. Butcher, to give unique collaboration in a field which is of great concern to both branches of medicine.

Most of the chapters have undergone minor revisions with updating of references. The chapters on wound healing, vessels, and the eye and its adnexa have been completely rewritten or represent additions to the volume, and contribute significantly to the value of this edition.

These additions account for much of the increased page content of the second edition, for there has been little expansion of other areas. However, numerous illustrations have been added and a few deleted; the illustrations are of somewhat better technical quality than in the original edition. Also contributing to the present volume is an improved layout, which makes for easier reading and far easier use as a reference. This volume should continue to enjoy the same popularity that has distinguished the first edition.

Diseases of Laboratory Primates. By Theodore C. Ruch. Ph.D. Price, $7.50. Pp. 600, with 116 illustrations. W. B. Saunders Company, 218 W. Washington Sq. Philadelphia 5, 1959.

This volume is the first of a proposed "Handbook of the Primates," to be published in four parts. Investigators who have sorely felt the need for a book of this magnitude will be fully cognizant of its importance. The "simian primate," in its relatively new role of laboratory animal, has had both health and welfare provided rather precariously by well-meaning investigators. The scientists have frequently had to spend as much time searching for source material in the care and diseases of their fellow primates as in the planning and execution of the experiments.

Dr. Ruch, whose extensive knowledge of the biology of primates has been demonstrated on at least three previous occasions, has obviated much of this difficulty. The rhesus monkey (Macaca mulatta) is dealt with in detail, with frequent pertinent observations regarding other "simian primates"; a term used by the author to indicate exclusion of man, lemurs, tarsiers, and tree shrews.

Major chapters consider the etiological, systemic, and/or symptomatic aspects of disease in a manner which will be helpful even for the nonpathologist. Further reading is directed by a selected bibliography after each chapter.

Subsequent volumes which will deal with "The Laboratory Primate," available normative data, and an extensive bibliography, 1939-1959, are eagerly anticipated: The need for them is certainly well defined.

Biopsy Manual. By James D. Hardy, M.D.; James C. Griffin Jr., M.D., and Jorge A. Rodriguez, M.D. Price, $6.50. Pp. 150, with 54 figures. W. B. Saunders Company, 218 W. Washington Sq. Philadelphia 5, 1959.

By some inexplicable quirk of logic, many surgeons relegate biopsy procedures to the category of minor and insignificant procedures and promptly delegate the task to the intern or the junior resident. Pathologists are only too well acquainted with the pitfalls of such assigning of responsibility and thus will be delighted with this handbook of biopsy technique. A biopsy, on which diagnosis and treatment commonly depend, is no better than the care and judgment experienced in the performance of that biopsy. This book presents the experience of the department of surgery at the University of Mississippi. The authors describe indications, techniques, hazards, and complications of the commonly used biopsies. As might be expected, major emphasis is on technique, and here lies the value of the volume for the pathologist's reference library. By training the house staff properly, the pathologist can save himself the ordeals of trying to interpret inadequate biopsies.

After first presenting general problems of biopsy procedures, this book systematically covers the various body areas for biopsy techniques. Thus, sternal-marrow, rib, and pleural biopsies are all considered in the chapter that includes lung and mediastinal-node biopsy. Various endoscopic techniques are all considered in a single chapter. Abdominal, breast, and skin biopsies are each particularly well presented. Reflecting the apparent interests of the authors, orthopedic and hematologic biopsies are almost totally neglected. After saying that iliac-crest biopsies have largely replaced sternal-marrow examination, the authors describe sternal puncture and totally ignore aspiration of marrow from the ilium. Splenic biopsy is not mentioned, an omission which indicates the author's acceptance of the widely held, though erroneous, opinion of the extreme danger of this valuable diagnostic procedure.

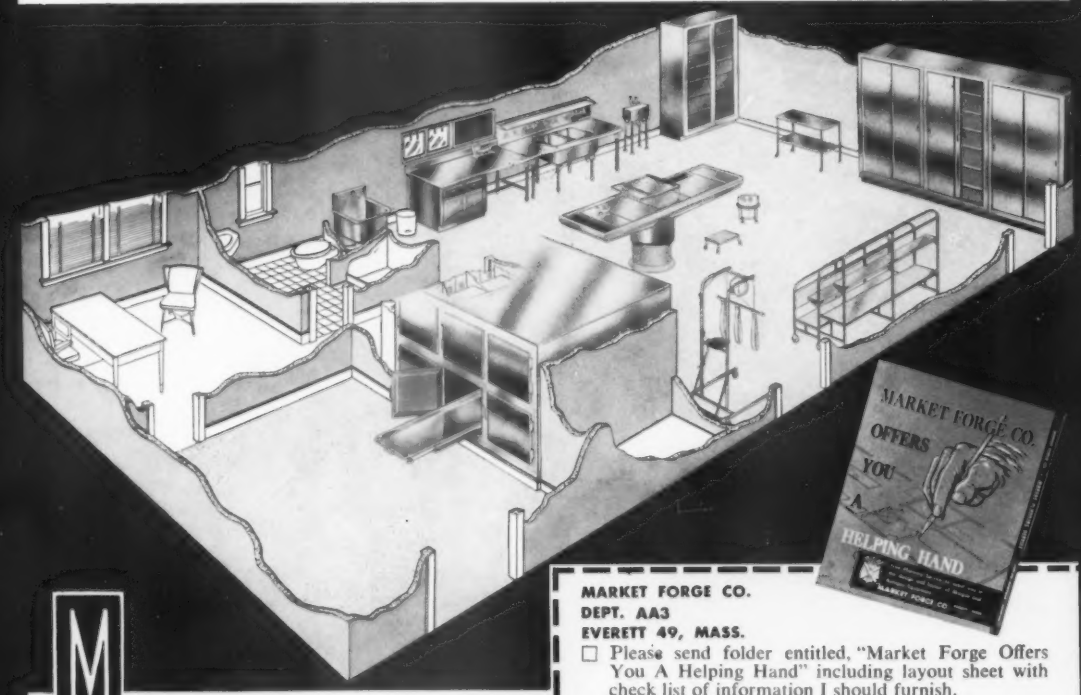
If one will accept other minor limitations, the volume is valuable to the pathologist as he instructs his surgical associates. The book is well written and beautifully illustrated with line drawings. While it presents often only one technique for a given procedure, it gives the reasoning for that technique and thus is a valuable guide. Throughout the book there is emphasis that the biopsy specimens must satisfy the requirements of the pathologist; such a concept should go far toward establishing understanding between the pathologist and the surgeon in this area where the patient particularly stands to benefit.

Market Forge offers you a helping hand...

Now Market Forge offers to pathologists, administrators, consultants and architects a free Morgue Planning Service to help you solve your morgue planning problems. Our staff of specialists will create an efficient, economical design and layout for morgue and autopsy equipment to fit your needs and budget. Here's what the free Planning Service will do . . .

- *help you achieve maximum utilization of equipment and available space.*
- *analyze operating functions and work flow.*
- *supply a scale drawing showing recommendations for equipment and its location.*
- *make revisions in accordance with your comments and suggestions.*
- *after layout has been checked and approved, Market Forge will add all necessary service connections and return the finished drawing to you.*

Simply send us a sketch of available areas or ask for our new folder entitled, "Market Forge Offers You A Helping Hand," which contains a layout sheet with a check list of information that you should furnish us.



MF CO.

HOSPITAL EQUIPMENT DIVISION

MARKET FORGE COMPANY

EVERETT 49, MASS. EST. 1897

MARKET FORGE CO.
DEPT. AA3
EVERETT 49, MASS.

☐ Please send folder entitled, "Market Forge Offers You A Helping Hand" including layout sheet with check list of information I should furnish.

☐ Have representative call.

☐ Send catalog on Autopsy Tables, Mortuary Refrigerators and other morgue equipment.

INSTITUTION .....

NAME ..... TITLE .....

ADDRESS .....

CITY ..... ZONE ..... STATE .....

first then...

Trailblazer in automatic tissue processing was this first Autotechnicon introduced in 1928. Primitive by today's standards (it held only six solution-changes) but what a revolution it brought in pathology laboratory procedure!

first now

Almost three decades of continuing improvement culminate in the new Autotechnicon "duo" ... first to combine separate processing and staining facilities on the same machine, with either available at the flip of a switch and without having to change beakers.

a name you can bank on for quality . efficiency . progress

the Autotechnicon®

trailblazer in histologic automation

THE TECHNICON COMPANY • CHAUNCEY, NEW YORK